CLINICAL AND EXPERIMENTAL STUDIES OF ^{67}GA-CITRATE AND OTHER TUMOUR IMAGING RADIOPHARMACEUTICALS

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ABSTRACT OF THESIS

^{32}P, ^{131}I-H.S.A., ^{75}Se-selenomethionine, ^{197}Hg-chlormerodrin, ^{67}Ga-citrate and labelled bleomycin compounds are discussed in a historical review. There are similarities in the tumours demonstrable by these agents and all of them may be taken up into inflammatory exudates, suggesting that, while differences in the metabolism of each agent exist, their mechanism of uptake may be predominantly non-specific.

Clinical studies have led to the following conclusions:

1). ^{67}Ga-citrate is a more useful tumour imaging agent than ^{111}In-bleomycin, for lesions above the diaphragm.

2). ^{67}Ga-citrate scanning is useful in the management of seminoma of the testis, giving consistently accurate tumour localization. Scans in patients with teratoma of the testis were less consistently positive. In patients with combined tumours (seminoma/teratoma), the scan seemed to reflect the dominant tumour type at the time of scanning.

3). A retrospective analysis of ^{67}Ga-citrate scans in patients with Hodgkin's Disease suggested that patients with lesions exhibiting high levels of ^{67}Ga uptake may have a more aggressive form of disease or may be in a more aggressive phase of their disease than patients with a low level of uptake; a prospective study is required for confirmation.

The mechanism of uptake of ^{67}Ga into tissues is discussed and was studied in several experiments. Comparative studies of the uptake of ^{67}Ga and ^{45}Ca in lactating and tumour-bearing dogs have shown that while there may be similarities in the metabolic pathways of gallium and calcium in the

lactating mammary gland, there is no similarity in the mechanism of uptake of these two elements into tumours.

Experiments using transplantable rodent tumours with variable percentages of tumour macrophages suggested that macrophages per se do not play a major role in ^{67}Ga uptake.

A preliminary study of ^{67}Ga and ^{125}I-labelled serum albumin suggested that tumour vascular permeability plays a role in the early accumulation of ^{67}Ga.

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INTRODUCTION

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1a). AIMS OF RADIOPHARMACEUTICAL TUMOUR IMAGING

In order to improve the treatment of malignant disease by surgery, radiotherapy or chemotherapy, we must necessarily devise more sensitive techniques to increase our diagnostic accuracy. The importance of a thorough and accurate pre-treatment staging work-up for patients with malignant disease is now widely accepted, and this can only result in more appropriate therapy for patients.

The development of a reasonably safe radioactive scanning agent localizing in malignant neoplasms in high concentration enabling a reliable and accurate mapping of the primary tumour, its local spread and distant metastases would be a very great advance in the management of malignant disease. If this agent were sufficiently specific for malignant neoplasms then a very useful aid to differential diagnosis would be available to us.

At the present time, however, radioactive substances which localize within tumours play only a limited role in the management of the cancer patient. This is because of the generally low tumour:background ratios achieved by currently available agents.

When a radiopharmaceutical is called "a tumour scanning agent" or a "tumour localizing agent" this implies not merely that the agent concentrates within a tumour but that it concentrates to a sufficient extent to enable a clinically useful scan to be produced using currently available instruments. We now have reached the stage when gamma-imaging instruments are extremely effective and further improvement in instrumentation is unlikely to offer much in the way of increasing our diagnostic efficiency. It is the concentration ratio of tumour:background tissue which determines whether or not a lesion can

be detected by radiopharmaceuticals. Typically when a tumour:background tissue ratio is 2:1, even a superficial lesion must be of the order of 2 cm. in diameter before it will be detected. Obviously, improvement of the concentration ratio of the agent will enable smaller lesions to be seen.

1b). EARLY TUMOUR IMAGING AGENTS

Even before the development of the high energy photon scintillation scanners in the late forties and early fifties, the idea of tumour localization by means of radioisotopes had received some attention. By and large, the techniques involved Geiger-Mueller counters used as probe detectors. When ^{32}P was introduced in 1936 and found to be effective in the treatment of the myeloproliferative disorders, much interest naturally arose as to its possible therapeutic effect in other neoplastic conditions. Disappointingly, the affinity of radioactive phosphorus for other types of malignancy was low except for brain tumours (Erickson, Larson and Gordon, 1948), but the concentrations achieved in the brain were of the same order as those occurring in other tissues with a rapid rate of phosphate metabolism such as the liver, and therefore, emphasis fell less on its therapeutic use in the brain and more on its diagnostic use as a tumour localizing agent. It found a limited clinical application in determining the extent of a brain tumour at operation (Stapleton, McKissock and Farran, 1952).

Similarly ^{131}I may be said to be an early tumour localizing agent. Known to localize in certain functioning thyroid carcinomas, particularly follicular carcinoma of the thyroid, ^{131}I was first used in the forties for the treatment of inoperable functioning carcinomas. The main problem was whether or not the tumour would take up the radio-isotope. Thus, often

these patients were given a tracer dose of ^{131}I, again detected by Geiger-Mueller counters (Pochin, 1956).

1c). GAMMA-EMITTING RADIOPHARMACEUTICALS

Further advances in tumour localization using radio-isotopes had to await the development of high energy scintillation detectors. These became generally available in the early fifties but for a number of years tumour delineation was limited to the uptake of ^{131}I functioning thyroid tumours, the outlining of brain tumours and the demonstration of filling defects in certain organs using various radio-active compounds.

1. RADIOMERCURY COMPOUNDS

In 1960, ^{203}Hg-chlormerodrin was introduced as a brain scanning agent. Later, ^{197}Hg-chlormerodrin was produced for brain scanning purposes and was a safer agent because of a much lower radiation dose to the kidneys. Curiously, it was not until four years later that Sodee, Renner and Di Stefano (1966) noticed that this agent concentrated in malignant tissues. They reported an impressive success rate particularly in malignant tumours of the lung, eye, nasopharynx, and in the lymphomas. Uptake was also present in a high percentage of patients with tumours of the pancreas, bowel, bone and breast although fewer cases of these were scanned. Interestingly, several false positives were encountered in the group of lung lesions and these eventually turned out to be cases of histoplasmosis and tuberculosis, causes of uptake which are not limited to ^{197}Hg-chlormerodrin or $^{197}Hg\ Cl_2$.

Subsequently many papers have appeared, particularly from France, reporting series of patients tested with ^{197}Hg-chlormerodrin or $^{197}Hg\ Cl_2$,

showing that good scans can be obtained in a variety of neoplasms (Rosenthall, Greyson, and Eidinger, 1970; Lamy et al., 1970). Indeed in many aspects the range of tumours having favourable uptakes are comparable to those obtained with ^{67}Ga-citrate, probably the most widely used of tumour scanning agents (see Table 1 p 6). Carcinoma of bronchus, secondary lung metastases and lymphomas generally show good uptake of radio-mercury compounds. For example, in carcinoma of the bronchus, of 217 cases reported in the world literature, 81% had positive scans, compared with 89% of 308 cases using ^{67}Ga-citrate. Again of interest in these papers is the incorporation of ^{197}Hg Cl_2 into various benign lesions which closely parallels the behaviour of ^{67}Ga-citrate. In a proportion of cases of sarcoidosis, abestosis, lung abscess, bacterial pneumonias and tuberculosis, uptake of ^{197}Hg Cl_2 occurs. The cases of sarcoidosis showing uptake are those patients with active disease, particularly if hilar lymph node involvement is present. In general, the chronic sarcoidosis patient with fibrosis has a negative scan. Issac et al., (1970) showed uptake in active tuberculosis but not in chronic tuberculosis. Bacterial pneumonias may also show uptake. These are all lesions which exhibit ^{67}Ga-citrate uptake.

The mechanism of uptake of radio-mercury compounds is unknown but very little research has been done on the subject. Biopsies from two cases of astrocytomas have been investigated using autoradiography, and accumulation of radioactivity was seen to occur on or within the neoplastic cells (Jackson, Carson and Dick 1967).

One serious disadvantage of radio-mercury compounds is the radiation dose to the kidneys. Even in the case of ^{197}Hg, this may amount to 5-13 rads when 10 Ci/kg body weight is administered.

TABLE I

Results of scanning with ^{67}Ga-citrate and ^{197}Hg-compounds

Disease	% positive scans using compounds containing ^{197}Hg	% positive scans using ^{67}Ga-citrate
Malignant melanoma	66% 20/30 (1)	66% 36/54 (13)
Hodgkin's disease	100% 2/2 (1)	82% 83/101 (23)
Non-Hodgkin lymphoma	66% 6/9 (3)	69% 38/55 (15)
Carcinoma bronchus	80% 163/203 (9)	89% 276/308 (12)
Mesothelioma	40% 2/5 (1)	60% 3/5 (3)
Carcinoid tumour	— 0/4 (2)	— 0/4 (4)
Pulmonary tuberculosis	44% 36/81 (7)	30% 19/62 (8)
Sarcoidosis	75% 3/4 (2)	89% 32/36

However, more fundamentally, although these radio-mercury compounds and ^{67}Ga-citrate appear to be taken up by the same kinds of lesions, the quality of scans obtained, reflecting the relative concentration of the agent within the tumour and the tissue background, is higher with ^{67}Ga-citrate than with ^{197}Hg-chlormerodrin or chloride, although no comparative clinical trials have been carried out.

2. ^{131}I-LABELLED COMPOUNDS

It is known that malignant tumours in experimental rats and mice take up larger amounts of unaltered plasma proteins from the circulation than do other tissues (Mego and McQueen, 1965). It is reasonable to expect that the same phenomenon may exist in man. What is known is that certain radio-iodinated protein compounds do localize in human malignant tumours and can be detected in sufficient concentration to produce reasonable scans. The compound most widely used in man has been ^{131}I-labelled human serum albumin. ^{131}I-labelled fibrinogen (Day, Pianisek and Pressman, 1959) and ^{131}I-labelled globulin (Hiramatsu et al., 1965) have been used in experimental animals in addition to ^{131}I-labelled albumin. Finney and his co-workers (1964) reported that ^{131}I-labelled albumin concentrated in the Walker 256 carcinoma in rats and the VX2 carcinoma in rabbits, but felt that this occurred only after intra-arterial perfusion of the tumour with hydrogen peroxide. The same group also studied ^{131}I H.S.A. uptake in human cancers and reported favourable concentration after perfusion, but poor concentration if peroxide was not perfused. Details of the controls used were not given in their paper, and shortly after it was shown that neither hydrogen peroxide nor intra-arterial administration seemed to be necessary for uptake of ^{131}I H.S.A. to occur. Bonte el al., (1966)

and Hisada, Hiraki and Ohba (1966) have both reported series of patients scanned using ^{131}I H.S.A. given intravenously. These scans are often of good quality and interestingly, there is a high success rate in gynecological cancers. This is a tumour type which has a disappointing success rate using ^{67}Ga-citrate and suggests more fundamental difference in the behaviour of the two agents towards gynecological tumours (see table 2). Bonte et al., (1966) have discussed the mode of uptake of ^{131}I H.S.A. based on work done on experimental rabbits using the V2 carcinoma and on studies of patients suffering from malignant disease. He suggested that the ^{131}I H.S.A. rapidly dispersed evenly throughout the blood pool of the tumour and normal structures. After 24 hours, secondary mechanisms come into play. There is a pooling of ^{131}I H.S.A. within the necrotic centre of a rapidly growing tumour. Since malignant cells have the property of pinocytosis of proteins, this may well account for the presence of the radio-isotope on or within the neoplastic cell. The same group also suggested that concentrations of tumour scanning agents might be improved by giving substances capable of increasing the permeability of the blood vessels of the tumour. The leakage of plasma proteins may also account for the positive scans seen in the cases of inflammatory and granulomatous exudate.

Fibrin is laid down around malignant tumours and especially rapidly growing ones. This had lead to the use of ^{131}I-labelled fibrinogen and ^{131}I-labelled anti-fibrin antibodies in experimental animals. There is certainly sufficient concentration of the compounds in the region of tumours to obtain scans. In some tumours, high tumour:background ratios are achieved but only limited trials have been conducted on patients (Day et al., 1959).

It might be worth looking at these compounds again from the point of view of scanning malignancies from the female reproductive tract.

3. ^{75}SELENIUM COMPOUNDS

^{75}Selenium labelled compounds have been used since the mid-sixties for the demonstration of certain tumours using the technique of whole body scanning, although following the introduction of ^{67}Ga-citrate in 1969, their use for this purpose has diminished considerably. They remain, however, an interesting group of compounds since reported clinical and experimental studies of their uptake into tumours provided a comparison to studies of the uptake mechanisms of the more recently introduced tumour imaging agents.

The first ^{75}Selenium compound to be used for tumour detection was ^{75}Se-selenite. Its introduction for this purpose was initially based on the hypothesis that because of similarities in the chemical properties of selenium and sulphur, tumours which laid down chondroitin sulphate (i.e. cartilaginous tumours) might be detected by incorporating a suitably labelled selenium compound. This, in fact, turned out to be correct (Esteban, Iasa and Perez-Modrego, 1965), but it was not realised at that time that the phenomenon of nuclide uptake into tumours was much more generalized than merely uptake into cartilaginous tumours. It was not until later when Cavalieri, Scott and Sairenji (1966), and Herrera et al (1966), more or less simultaneously reported their observations on ^{75}Se-selenomethionine respectively that this became apparent. The former group based their clinical study on observations made in sarcoma bearing rats and found that a variety of tumours could be detected using ^{75}Se-selenite. These tumours included cerebral astrocytomas, bronchogenic carcinomas, a case of myeloma and a case of carcinoma of the colon. These investigators felt that ^{75}Se-selenite was more tumour-specific than any agent previously studied, since cases of cerebral infarction, lung abscess and pneumonia which they

COMPARISON OF SCANNING SUCCESS RATES BETWEEN ^{131}I-HUMAN SERUM ALBUMIN AND ^{67}GA IN THREE GYNAECOLOGICAL MALIGNANCIES

	^{131}I-H.S.A.	^{67}Ga
Carcinoma cervix	100% 8/8 (1)	16% 2/12 (4)
Carcinoma ovary	66% 2/3 (2)	33% 3/9 (3)
Carcinoma uterus		25% 1/4 (2)

examined showed no significant uptake on scanning. Baptista (1972), has since studied 58 patients with a variety of hepatic lesions by comparative scintigraphy using ^{198}Au or $^{99}Tc^{m}$ colloid and ^{75}Se-selenite. He has supported the suggestion that ^{75}Se-selenite is more tumour-specific than other agents. Of 15 patients with benign lesions of the liver (e.g. abscesses, cysts, cirrhosis) all gave negative scans, whereas the remaining 43 patients with primary or metastatic liver disease all exhibited positive uptake of ^{75}Se. Esteban et al., (1972) reported very encouraging but less specific results with ^{75}Se-selenite for lesions in the same area. However, there has been little further confirmatory work reported in the literature on this aspect of ^{75}Se-selenomethionine.

While scanning the pancreas of a patient with ^{75}Se-selenomethionine, Herrera et al., (1966) observed heavy uptake in a retro-peritoneal mass of lymphosarcoma. These workers then went on to examine a further 9 patients with a variety of lymphomas and recorded a gratifying success rate. They also observed that radiotherapy very quickly caused the increased uptake to return to normal. However, again, it was not appreciated that ^{75}Se-selenomethionine was taken up by a wider range of tumours than the lymphomas they had examined, and it was not until further studies were published that this became apparent. D'Angio, Loken and Nesbit (1969) showed that neuroblastoma in children could be scanned successfully and hepatomas have also be recorded as exhibiting good ^{75}Se-selenomethionine uptake (Stolzenbert, 1972). Kaplan and Domingo (1972) found the diagnostic value of liver scanning could be enhanced by using a dual-channel subtraction technique with $^{99}Tc^{m}$ sulphur-colloid and ^{75}Se-selenomethionine. They correlated ^{75}Se uptake with the vascularity of various types of lesions as demonstrated by hepatic angiography. Thus, the relatively avascular amoebic

abscesses and metastases from tumours of the bronchus or pancreas showed little uptake. It is interesting to note that these results are broadly similar to those obtained by Lomas, Dibos and Wagner (1972) and Suzuki, Honjo and Hamamoto (1971) who used ^{67}Ga-citrate to demonstrate hepatic lesions. It may be added in parenthesis here that the importance of isotopic methods in the differential diagnosis of liver disease is likely to decline somewhat with the development of grey-scale ultrasonic scanning of this area. Thomas, Pepper and Owen (1969) showed that uptake occurred not only in malignant thyroid lesions but also to a lesser extent in benign thyroid lesions.

In a study designed to assess the practical value of scanning with ^{75}Se-selenomethionine in lymphomas and seminomas Ferruci, Berke and Potsaid (1970) compared the abdominal findings in patients with lymphoma using X-ray lymphangiography and isotope scanning. In fact, the ^{75}Se-selenomethionine method compared quite favourably with X-ray lymphangiography although in one third of positive lymphangiograms, the scans were negative. Conversely, however, the scan was positive in 25% of cases where the X-ray lymphangiography was difficult to interpret or reported as normal. Some of these may have been false positives but the majority indicated disease not initially detectable on the X-ray. One especially interesting point in this study was the report of extremely good results in the scanning of seminoma of the testis; this is a tumour we ourselves have found to be very satisfactorily scanned with ^{67}Ga-citrate. (Paterson, Peckham and McCready, 1976).

Studies of mechanisms of uptake of ^{75}Se-selenomethionine provide interesting reading. As mentioned above, the clinical studies on hepatic lesions seemed to indicate some relationship between uptake of ^{75}Se-selenomethionine and tumour vascularity. This aspect has not been adequately examined

in animals and must remain in open question. However, the striking similarities in the nuclide uptake characteristics of various lesions between ^{75}Se-selenomethionine and ^{67}Ga-citrate argue strongly in favour of the suggestion that tumour blood vessel permeability may play an important role in the uptake of many agents into tumours.

Since ^{75}Se-selenomethionine is a labelled essential amino-acid, it is not unreasonable to postulate that increased rate of protein synthesis within tumours is a cause of its concentration, and the contribution of protein synthesis to the uptake of ^{75}Se-selenomethionine has indeed been studied in some detail. It has been well shown that certain tumours among which are included lymphomas and seminomas, have a more rapid rate of protein turnover than have normal tissues (Quastel and Bickis, 1959). Spencer et al (1967) studied the incorporation of ^{75}Se-selenomethionine into tumours. ^{75}Se uptake into protein increased steadily with time in both tumour and liver reaching 40% of the total tumour and liver ^{75}Se at one hour after injection. The concomitant administration of inhibitors of protein synthesis (Actinomycin D or Puromycin) lowered the amount of ^{75}Se which was protein-bound, although whether or not the total tumour uptake was affected is not mentioned.

Although the biochemical and nutritional aspects of selenium metabolism have been the subject of considerable study, mechanisms of uptake into tumours other than the possible relationship to protein metabolism outlined above have not been examined to any great extent. Selenite is a potent oxidiser of sulphydril groups (Painter 1941), and this is a reason for its rapid fixation in normal tissues, and may account for its accumulation in tumours as well. Another suggested possibility (Shrift, 1961) is that selenite is metabolised

by intestinal flora to selenomethionine in which form it might enter the tumour.

In the last few years other agents such as ^{67}Ga-citrate and labelled bleomycin compounds have taken over from ^{75}Se labelled compounds as the tumour imaging agents of choice. There have been no direct comparative studies of these agents with ^{75}Se-selenomethionine, although the clinical impression is that the more recent agents have more favourable tumour:background radioactivity ratios. The greatest disadvantage of the ^{75}Se labelled compounds is their long biological half life, which in the case of ^{75}Se-selenomethionine is 45 days, and ^{75}Se-selenite is 65 days.

In conclusion, I would like to draw attention to some of the striking similarities in the medical conditions producing sufficient uptake to give scintiscans with ^{75}Se-selenomethionine and ^{67}Ga-citrate, for exampie, lymphomas, Ca. bronchus, seminomas, and hepatomas. These similar conditions inevitably lead one to postulate that there may be similarities in mechanisms of uptake of some of these compounds. That there are secondary mechanisms occurring is well illustrated by the experimental studies described above, but whether these mechanisms are the most important from the point of view of tumour imaging, remains to be seen.

4. ^{67}GA-CITRATE

^{67}Gallium is a cyclotron produced radionuclide, which is usually produced as ^{67}Ga-citrate by the addition of 3.8% sodium citrate. It has a physical half-life of three and a quarter days, and the radiation received by the patient is less than 1 rad to the whole body and less than 5 rads to the kidneys.

In the fifties, other isotopes of Gallium, particularly ^{68}Ga and ^{72}Ga had been investigated as possible new agents for the study of normal and neoplastic bone metabolism (Dudley, Henry and Lundsly, 1950). It was when ^{67}Ga was being similarly investigated that its uptake was observed in a group of lymph nodes in a patient with Hodgkin's Disease (Edwards and Hayes, 1969). When reported, this observation initiated a great deal of work in both the clinical and scientific spheres mainly directed to defining the value and specificity of ^{67}Ga as a tumour localizing agent and explaining the mechanism of uptake.

^{67}Ga localizes in many kinds of tumour. However, the concentration achieved may be insufficient for scanning purposes particularly in small lesions. A good example of this occurs in tumours of the stomach and oesophagus where uptake can be demonstrated in resection specimens but a clinically useful scan is rarely achieved. Higasi et al., (1971) found that, in four cases of carcinoma of the stomach, three cases were negative and one case was inconclusive on the clinical scans. However, the radioactivity in the neoplastic areas of the resected specimens was 2.0-3.2 times greater than that of the unaffected surrounding tissue ofthe stomach. Colonic and rectal cancers have also been investigated from this point of view and despite negative scans, a good concentration has been measured in the malignant tissue in operative specimens. Therefore, it seems likely that ^{67}Ga concentrates in many different varieties of malignancy and more consistently than clinical scanning success rates suggest. Reasons for failure to detect these lesions may be poor scanning technique; difficulties in differentiating normal uptake from abnormal uptake (for example in the region of the liver or where it has been difficult to completely clear the bowel of faeces) and low tumour:background ratios. It seems unlikely that

further improvement in instrumentation will help in these areas and efforts should be devoted now to improving the relative concentration of ^{67}Ga within malignant lesions. We know that if a tumour:background tissue uptake ratio of 2:1 is present, a lesion must be at least 2 cm. in diameter before it will be detected. This is often the kind of ratio that is achieved in malignancies of the gastro-intestinal tract and may partially explain the low scanning success rates in such tumours.

However much experience has been gathered in the last few years in the use of ^{67}Ga-citrate. It has become apparent that certain malignancies are more rewarding than others in terms of acquiring clinically useful scans. Lymphomas, particularly Hodgkin's Disease and histocytic lymphomas, have a high success rate (Edwards and Hayes, 1970; Langhammer et al., 1972; Berelowitz and Blake, 1972). Kay and McCready (1972) at the Royal Marsden Hospital, and Turner et al., (1972) have examined the place of ^{67}Ga scanning in the management of patients with Hodgkin's Disease. Both groups found that there was a 70% detection rate in areas of active disease. Kay and McCready, in addition pointed out that the technique was useful in detection of mediastinal disease. They cited six patients with normal chest x-ray where ^{67}Ga showed increased uptake in the mediastinum, who subsequently went on to develop clinically overt Hodgkin's Disease. They also pointed out the value of ^{67}Ga-citrate in the differentiation of fibrosis from recurrent tumour.

Bronchogenic carcinomas (Higasi et al, 1971; Ito et al., 1971; Muhe, 1971), hepatomas (Higasi et al., 1971; Suzuki, Honjo and Hamamoto, 1971; Lomas, DiBos and Wagner, 1972; Winchell et al., 1970) osteosarcomas

(Okuyama et al., 1972; Berelowitz and Blake, 1972), carcinomas of the maxilla (Higasi et al., 1971; Berelowitz and Blake), all show consistently high scanning success rates. Ito et al., (1971) stated that in carcinoma of the bronchus, ^{67}Ga scanning was very useful for the detection of infraclavicular nodes difficult to palpate clinically, and mediastinal metastases difficult to demonstrate radiologically, while Mühe (1971) suggested that this was the simplest method for detecting recurrences and was more accurate in differentiating a malignant from a benign tumour than bronchoscopy, mediastinoscopy or sputum examination.

Investigating over 60 patients who had focal defects on colloid liver scans Lomas et al. (1972) found that increased specificity of diagnosis was achieved when ^{67}Ga scanning was also performed. The physiological uptake of ^{67}Ga over the liver makes interpretation in this area difficult on ^{67}Ga scanning alone, while the two isotopes together (^{67}Ga and ^{99m}Tc) helped to differentiate cancer and abscesses from more benign causes of an abnormal liver scan. In particular, cirrhotic livers giving "patchy" colloid scans where doubt existed as to the possibility of development of a hepatoma, could be investigated with ^{67}Ga, a localized area of high uptake being highly suggestive of hepatoma. Hepatomas, indeed, invariably show increased ^{67}Ga uptake, in contrast to intrahepatic secondary deposits, which are often not demonstrable. Only four of nine patients with liver metastases from an adenocarcinoma showed uptake in Lomas's series. An explanation for this was put forward by Suzuki et al., (1971) who reported the same phenomenon. In an elegant study, this group performed selective hepatic arteriography on 25 patients with liver cancer. Radioactive gold-colloid and ^{67}Ga scans were done on the same patients. They found that the highly vascular hepatomas invariably concentrated ^{67}Ga, whereas the avascular secondary

metastases showed little concentration. Those secondaries with moderately well developed tumour circulations showed moderate ^{67}Ga uptake.

$^{99}Tc^{m}$ pertechnetate is probably the most suitable agent for routine scanning of suspected intracranial lesions, but there are occasions when ^{67}Ga-citrate brain scanning can give additional information as outlined by Jones et al., (1972). One third of the cases in this series were more clearly shown using ^{67}Ga-citrate, but most usefully, $^{67}Gallium$ did not appear to be taken up by the craniotomy bone-flap, as is $^{99}Tc^{m}$ pertechnetate. Gallium scans, therefore, can aid in the differentiation of recurrence of residual tumour from uptake at the operative site. Interestingly, the Bethesda group also described two cases, one a brain metastasis from a bronchial adenocarcinoma and the other a glioblastoma multiforme, both of which had received radiotherapy or chemotherapy very shortly before the scans were done. The ^{67}Ga scans showed poor uptake whereas the $^{99}Tc^{m}$ scans clearly imaged the tumour. The implication of this is, of course, that shortly after treatment the vascular space within the tumour was unchanged but that the treatment interfered with the mechanism of uptake of ^{67}Ga. It has frequently been demonstrated that ^{67}Ga uptake decreases to normal levels after successful treatment of an involved area and it may be that ^{67}Ga studies will provide a sensitive and early indicator of effective treatment.

Less consistently successful scans are obtained in malignant melanoma (Berelowitz et al., 1972; Lavender et al., 1971; Langhammer et al., 1972; Milder, 1973) hypernephroma (Berelowitz and Blake, 1972; Antoniades et al., 1973), carcinomas of the breast (Lavender et al., 1971; Fogh and Edeling, 1972), colon and rectum (Nash et al., 1972; Langhammer et al., 1972), and thyroid (Edwards and Hayes, 1970; Langhammer et al., 1972).

Intra-ocular melanoma deposits have given particularly disappointing detection rates. Heuer, Ehlers and Hansen (1972) examined nine patients with malignant melanoma of the choroid. ^{67}Ga accumulation did not occur in any of the cases. These were, however, very small tumours most having a range of diameters from 0.5-1.5 cm. He concluded rightly that for ophthalmologists "the method does not in its present form offer any clinical information."

Very variable results have been reported in ^{67}Ga scanning of carcinoma of the breast. Ito et al. (1971) have reported a good scanning success rate, while others have had singularly poor results. Lavender et al. (1971) examined 25 patients with carcinoma of the breast. Of 12 patients with operable primary tumours, none gave positive scans. Nine of 13 patients with metastatic or advanced lesions gave positive scans. Here again, tumour size may have been important in the detection of these lesions. This group reported uptake in breast abscess.

Colonic and rectal tumours have been observed by various investigators to concentrate ^{67}Ga sufficiently to produce a positive scan in approximately 40% of cases. Normal faecal excretion of the isotope makes bowel washouts necessary if good scans are to be obtained. As has been previously mentioned, although this is rather a low clinical success rate, it must be compared to increased tumour:background ratios which occur far more frequently than the detection rate suggests. Nash et al. (1972) examined the uptake of ^{67}Ga in 20 resection specimens of colonic and rectal disease, predominantly malignancies. In 13 out of 15 malignancies the tumour:normal tissue ratio was greater than 2:1. In five of the tumours, the ratio was greater than 5:1. An attempt was made to correlate the pathology of the tumours with the

uptake. The tumours were graded as poorly, moderately or well differentiated. The degree of differentiation of a tumour only implies that a growth rate is fast or slow, and mitotic indices or tritiated thymidine labelling indices were not done in this study. However, generally it was found that the poorly differentiated tumours took up ^{67}Gallium more avidly than either the moderately or well differentiated tumours, and the moderately differentiated ones took up slightly more ^{67}Ga than the well differentiated ones. The ^{67}Ga activity in cross-sections of the growths was measured and the greatest activity was found in the actively growing tumour edge while the necrotic centre showed low uptake. This parallels other observations that ^{67}Ga localizes in viable tumour cells and not in necrotic tumour (Hayes, Nelson and Swartzendruber, 1970). Suprisingly, there have been no more detailed studies of ^{67}Ga uptake and the degree of tumour differentiation and growth rate in human cancer although several attempts to correlate ^{67}Ga uptake with histological tumour cell types have been made without success (Van der Schoot, Groen and Jong, 1972; Siersbaek-Neilson, Hansen and Fris, 1972).

There is no doubt that ^{67}Ga scanning in its present form offers little clinical help in gynaecological malignancies and carcinomas of the oesophagus, stomach and pancreas (Higasi et al., 1971). As has been said before, concentration occurs but it is often insufficient for scanning purposes.

Hopes that ^{67}Ga-citrate was a truly specific malignant tumour scanning agent were unfounded when reports began to be published showing uptake in a variety of lesions. It has been demonstrated that ^{67}Ga is taken up by abscesses in a variety of sites. These included breast (Lavender et al., 1971), ovary (Symmonds and Tauxe, 1972), gallbladder (Lomas and Wagner, 1972) and psoas muscle (Fratting and Sharp, 1973). Lomas and Wagner (1972) suggested

that since the uptake of ^{67}Ga had been observed in four consecutive cases of gallbladder empyema, ^{67}Ga scanning might be of value in the pre-operative diagnosis of inflammatory gallbladder disease since a negative oral cholecystogram is a non-specific finding. Geloud, Arseneau and Johnston (1973) examined uptake of ^{67}Ga in experimental sterile and infectious abscesses and found on autoradiography of the abscess aspirates, that radioactivity was confined largely to the "inflammatory" cells.

Other inflammatory lesions which have been recorded as giving positive scans are pneumonia both bacterial and viral(Kinoshita et al., 1973), tuberculosis (Dige-Petersen, Heckscher and Hertz, 1972) and subacute thyroiditis (Grove, 1973). The cases of tuberculosis which concentrate ^{67}Ga are generally those of "active" disease, chronic cases on the whole showing no uptake. Active sarcoidosis invariably concentrates ^{67}Ga, to the extent that ^{67}Ga scanning could be performed as a monitor of disease progress.

It is my own and others experience that ^{67}Ga does not concentrate within post-irradiation fibrosis. This is very useful in the differential diagnosis of tumour recurrence from post-treatment fibrosis. However, in other fibrotic diseases, such as silicosis, there is often a diffuse, light uptake (Winsor et al., 1973).

Investigation of the mechanism of uptake of ^{67}Ga by tumours and other tissues together with clinical studies will form the major part of this thesis.

1d). RECENTLY DEVELOPED TUMOUR IMAGING AGENTS

1. LABELLED BLEOMYCIN COMPOUNDS

Radionuclides chelated by bleomycin have been used widely in the last few years. Bleomycin is a polypeptide antibiotic with cytotoxic effects

(Umezawa et al., 1966; Tukeuchi and Yamamoto, 1968). Its distribution has been studied in animal tissues and it has been found to concentrate in skin, peritoneum, and to a lesser extent, lung tissue (Umezawa et al., 1968). Limited work has been done on its distribution in tumour-bearing mice, and it has been found to concentrate well in chemically induced squamous carcinomas of the skin and to a much lesser extent in skin sarcomas and brain gliomas (Umezawa et al., 1968; Hayakawa et al., 1974). Clinically, it has been found to have some therapeutic effect in certain cancers, particularly squamous carcinomas of the skin (Blum, Carter and Agre, 1973), and testicular tumours.

The first radionuclide chelated to bleomycin to be used in humans was ^{57}Co. Nouel and his colleagues (1972) examined ten patients suffering from a variety of malignant diseases and found that good scans were obtained in nearly all the patients. ^{57}Co-bleomycin has been shown to concentrate more favourably in experimental tumour-bearing animals than either ^{62}Zn-bleomycin (Taylor and Cottrall, 1973), $^{99}Tc^{m}$-bleomycin (Lin, Goodwin and Kruse, 1974), ^{67}Cu-bleomycin (Hall and O'Mara, 1974) and ^{111}In-bleomycin. The last of these has probably been the most extensively investigated. ^{111}In has a suitable half-life (67.5 hours) for use in patients, and emits two easily detectable spectrons of gamma radiation in cascade in abundance (171 keV 89% and 247 keV 94%).

Several clinical series have now been reported using this agent and although some of the reports are enthusiastic, it appears that this agent does not offer any real advance over ^{67}Ga-citrate in terms of improved tumour:background ratio. However it must be said that the faecal and small bowel uptake of ^{67}Ga can make interpretation of abdominal scans difficult and ^{111}In-bleomycin scans of the abdomen are much more easily read because of the rapid excretion of ^{111}In by the kidneys.

Lilien and his colleagues (1974) felt that the agent offered a significant advance in tumour diagnosis by demonstrating a wider range of

tumours than ^{67}Ga-citrate. They describe good uptakes in lymphomas and carcinomas of the bronchus and colon, but striking results were obtained with ovarian carcinomas (88% successful imaging) and melanomas (100% successful imaging). These tumours tend to be poorly demonstrated by ^{67}Ga-citrate. It is possible that Bleomycin's peculiar property of concentrating in skin and peritoneum has influenced the uptake of the agent in these cancers. The agent, again, is not specific for malignant lesions, positive scans having been recorded in a variety of inflammatory diseases. In the studies published, there are unfortunately no control scans with which to compare the agent being tested. Useful information in the clinical field can only be acquired by comparing any new agent with the best currently available and consequently a clinical comparative study of ^{111}In-bleomycin and ^{67}Ga-citrate will be described (Paterson, Taylor and McCready 1975) in a following section.

The metabolism of ^{111}In-bleomycin has been studied by Goodwin et al. (1974) among others. The kidneys excreted 70-80 per cent of the agent in the first 24 hours in humans. The remainder had a long biological half-life, with increasing marrow acitivity, which suggested that ^{111}In-bleomycin, initially tissue fixed, was being broken down and ^{111}In-transferrin was being transported to the bone marrow.

Nevertheless, ^{111}In-bleomycin represents a deliberate approach to the problem of tumour imaging by attempting to increase tumour uptake using a cytotoxic agent and to decrease tumour:background activity by modifying the mode of excretion of ^{111}In.

2. RADIOLANTHANIDES AND OTHERS

These rare earth elements of high atomic number have been found to concentrate within tumours by Hisada and Ando (1973). They have recently investigated 15 patients using ^{169}Ytterbium-citrate with good scanning results

in primary liver and lung cancers, lymphomas and bone metastases (Hisada et al., 1974). However, these are the kind of tumours which may be successfully imaged using ^{67}Ga-citrate. The tissue and subcellular distribtuion of the radiolanthanides has been compared to ^{67}Ga-citrate by Hayes et al., (1974). They found that these agents had comparable tumour localizing ability and a similar subcellular distribution to ^{67}Ga-citrate, and furthermore, tended to concentrate at site of inflammation. This would suggest that in tumour imaging, the radiolanthanides do not offer much more than ^{67}Ga-citrate.

Recent approaches to the problem of tumour imaging have included the tagging of tetracycline with ^{131}I (Chauncey, Halpern and Alazraki, 1974) and the use of labelled metabolites tailored to the particular tumour being investigated (Forman et al., 1974). A most interesting approach to the problem has been the labelling of antibody to carcino-embryonic antigen with ^{131}I (Hoffer et al., 1974). This may be useful in the diagnosis of gastro-intestinal tract tumours although high levels of circulating C.E.A. may well be a problem.

However, in ordinary clinical practice, most departments use ^{67}Ga-citrate for routine tumour imaging and this thesis will deal predominately with the clinical significance and mechanisms of uptake of ^{67}Ga-citrate in malignant tumours.

2. CLINICAL COMPARATIVE STUDIES OF TUMOUR IMAGING RADIOPHARMACEUTICALS

1. A Clinical Comparison of the Tumour Imaging Radiopharmaceuticals ^{67}Ga-citrate and ^{111}Indium-labelled bleomycin.

The main part of this section was published in the British Journal of Radiology, 48, 832-842 (1975)

1. INTRODUCTION

Clinical studies of tumour imaging agents have concentrated mainly on the question of the tumour specifity of the agent under investigation. Papers have tended to focus on whether or not a known malignant lesion, easily demonstrable by other means, could be demonstrated by scanning. In other words, it would appear that to many investigators, the most attractive features of these agents is their potential value in differential diagnosis. However, it has been shown time and time again that these agents have the property of uptake in a certain proportion of inflammatory and granulomatous disorders. We feel, therefore, that their value in differential diagnosis must be limited to situations where inflammation can be reasonably excluded, such as the differentiation of post-irradiation fibrosis from recurrence of tumour in the lung. A gallium scan under these circumstances can be most rewarding clinically.

Thus, clinical interest is likely to turn to other advantageous features of this diagnostic method, and one of the most obvious is the case in which the whole body may be scanned for the staging of a proven malignant lesion. To this end, improvement in tumour:background ratios must be sought.

Bleomycin is a polypeptide antibiotic with cytotoxic properties and is effective in the treatment of certain squamous carcinomas in animals and man (Umezawa et al., 1966; Takeuchi and Yamamoto, 1968; Blum, Carter and Agre 1973) and testicular tumours (Samuels et al., 1975). Studies of its distribution in mice have shown high concentrations of the drug in skin, peritoneum and, to a lesser extent, lungs (Umezawa, Ishizaka and Hori, 1968), but there is only limited evidence for its actual concentration within tumours. Uptake measurement of ^{3}H-bleomycin into squamous carcinomas of skin and skin sarcomas in mice have shown that concentration occurs in the carcinoma to a much greater extent than in the sarcoma (Umezawa, 1973). Using ^{14}C-bleomycin, Hayakawa et al., (1974) demonstrated tumour: normal tissue ratios of 3:1 in chemically induced gliomas in mice. No other tumours in animals or man have been examined.

The drug has chelating properties, thereby facilitating radiopharmaceutical production. Using ^{57}Co-labelled Bleomycin, Nouel, Renault and Robert (1972) studied ten patients with a variety of malignancies and found that this agent gave good scans in all the patients studied. However, the long half-life of ^{57}Co (270 days) is a disadvantage and consequently other more suitable radionuclides have been investigated. These have included ^{99}Tc^{m} bleomycin (Lin, Goodwin and Kruse, 1974), ^{62}Zn-bleomycin (Taylor and Cottrall, 1973), ^{67}Cu-bleomycin (Hall, O'Mara and Cruz, 1974) and ^{111}In-bleomycin.

Because of its more favourable physical characteristics, ^{111}In-bleomycin has been widely used as a tumour imaging agent and many reports have been published. The only way to properly assess this

new agent was to compare it in the same patient with the currently most widely used tumour imaging agent, ^{67}Ga-citrate. This study, therefore, reports the results of a comparative trial of ^{111}In-bleomycin and ^{67}Ga-citrate.

METHODS

This comparative study was divided into two sections. One part dealt with the relative scanning qualities of ^{111}In-bleomycin and ^{67}Ga-citrate and the other was a quantitative study of the relative uptake of the two radionuclides in biopsy samplings removed at operation.

In the first part of the study, 25 patients received both agents. Of these, one patient was examined with the two agents on three separate occasions and one patient was examined twice. Therefore, in all 28 examinations were performed. Whole-body scanning, using both agents, was performed to fully establish the total extent of disease. In those in whom scanning was of academic interest only, informed consent was obtained.

Eighteen of the 26 patients were examined during the initial period of investigations prior to treatment. In ten examinations, treatment with either chemotherapy or radiotherapy had to be given between scans. These cases will be dealt with separately since the possible effect of treatment on uptake requires individual assessment.

The diseases of the patients studied were: Hodgkin's disease (13 examinations), carcinoma of the bronchus (4 examinations), seminoma testis (3 examinations), non-Hodgkin's lymphomas (2 examinations) and osteosarcomas (2 examinations). One case each of malignant melanoma, myeloma, hydrocoele and carcinoma of the ovary were also examined.

The scans were performed as far apart from each other as was reasonably possible in order to diminish any possible effect one agent might have on the mechanism of uptake of the other. Nevertheless, in 13 examinations, the time interval between scans was only one week. Here, it is possible that the y-emission from the first agent could contribute to the scan obtained after the second agent had been given. In these 13 examinations, ^{111}In-bleomycin was the first agent given on seven occasions followed by ^{67}Ga-citrate. When ^{67}Ga-citrate was given before the ^{111}In-bleomycin, it was relatively easy to exclude any effect the previously administered ^{67}Ga-citrate might have on the ^{111}In-bleomycin scan by using the 147 keV peak of ^{111}In with a narrow window setting of 230-270 keV. However, it is not possible to exclude previously administered ^{111}In by using a selective window setting. Under these circumstances, a scan was performed prior to administration of the ^{67}Ga-citrate to make sure that an increase in tumour:background ratio after the 48 hours had not occurred, since this might have caused enhancement of the ^{67}Ga image. It must be added that this would be a

most unusual effect, and, furthermore, since three half-lives of ^{111}In decay had already occurred it would be most unlikely that the energy emitted from the ^{111}In would affect the scan obtained with the ^{67}Ga. Under these circumstances, no increase in tumour: background ratios was observed to occur over the week following the administration of ^{111}In-bleomycin and therefore these scans are directly comparable. Eleven examinations were performed with two weeks interval between scans (on six occasions ^{67}Ga-citrate was given first, and on the remaining five, ^{111}In-bleomycin was given first), and the remaining five examinations were conducted at three or more weeks interval between scans.

Carrier-free ^{111}In-bleomycin (bleomycin concentration 1 mg ml^{-1}) was obtained from the M.R.C. Cyclotron Unit, Hammersmith Hospital and latterly, from the Radiochemical Centre, Amersham, when the bleomycin concentration in the ^{111}In-bleomycin was 0.66 mg ml^{-1}. In both types there was approximately 1mCi of ^{111}In per ml. of isotonic saline, and therefore, the ^{111}In-bleomycin from the M.R.C. Cyclotron Unit contained more bleomycin per unit of radioactivity than the ^{111}In-bleomycin from the Radiochemical Centre.

It is possible that slight differences in rates of excretion of ^{111}In-bleomycin from the different suppliers might be observed, although it was not felt to be of much significance in this study. It was administered intravenously in doses ranging from 1-2.3 mCi depending on how much isotope was available each week. Most patients received approximately 2 mCi.

Carrier-free ^{67}Ga-citrate was obtained commercially from Philips-Duphar and was administered intravenously in doses ranging from 1.5 to 3.2 mCi, most patients receiving 2.5 mCi. Scans were performed on a Selo Superscanner D.S.7. forty-eight hours after injection of either radiopharmaceutical.

The second part of the study was concerned with the direct measurement of the uptake of the two agents in biopsy specimens removed at operation. Thse specimens were mainly lymph nodes and spleen (some specimens involved with lymphoma, others normal) from patients undergoing staging laparotomies for Hodgkin's disease or non-Hodgkin's lymphomas. Approximately 500 μCi of ^{111}In-bleomycin and 500 μCi of ^{67}Ga-citrate were given together intravenously 24 hrs. before operation. Blood samples were taken at 1, 3, and 24 hours after the injection. At 24 hours, the biopsy was performed and specimens were taken immediately for measurement of the relative uptake of ^{111}In-bleomycin and ^{67}Ga-citrate.

The samples of whole blood plasma and tissue were assayed for ^{67}Ga and ^{111}In in order to ascertain the range of concentrations occurring within the tissue.

RESULTS

The results of 28 separate examinations in 25 patients are given in Tables 2.1 and 2.2. Table 2.1 lists the results of those examinations performed during the initial diagnostic work-up. Table 2.2 lists the results of examinations performed where treatment has been given at some time before or between the scans.

The term "clearest image" is self-explanatory, the clearest images having the highest lesion:background radioactivity ratios. Obviously, the clearer the image, the lower the number of false negative interpretations. It is of course necessary to ascertain that the images obtained did actually delineate tumour, and therefore correlation with clinical findings was assessed. Because of the variable number of disease sites in an assortment of different tumours, there were some difficulties in grading clinical correlation. However, it was finally decided to use four broad assessment categories. These were:

GOOD - tumour imaging coincided with eventual clinical and pathological staging;

FAIR - if one site of disease was missed or was equivocal;

POOR - if no abnormal uptake occurred in the presence of known disease.

In 14 separate examinations, the ^{67}Ga-citrate scan was superior to the ^{111}In-bleomycin scan. There were no examinations in which the ^{111}In-bleomycin scan was judged to be superior to the ^{67}Ga-citrate scan.

TABLE 2.1
UNTREATED PATIENTS

No.	Name	Disease	Clinical features and treatment	Relative uptake of ^{111}In-bleo and ^{67}Ga												Clinical correlation		Best tumour-imaging agent
				Mediast.		Lung		Neck		Axilla		Abdo.		Other				
				In	Ga	In	Ga	In	Ga	In	Ga	In	Ga	In	Ga	In	Ga	
1	D.S.	Hodgkin's disease	Stage IIA (right neck, paratracheal and internal mammary nodes)	±	+			+	+					−	−	Poor	Fair	^{67}Ga
2	R.H.	Hodgkin's disease	Stage IIA (right neck and axillary nodes)					−	−	−	±					None	Poor	^{67}Ga
3	T.R.	Hodgkin's disease	Stage IA (left neck)					−	+							None	Good	^{67}Ga
4	A.W.	Hodgkin's disease	Stage IIE (left hilum and supraclav.)	±	+			±	+							Fair	Good	^{67}Ga
5	E.W.	Hodgkin's disease	Stage IV (mass of nodes on shoulder and infiltration of right lung)			−	+							+	+	Fair	Good	^{67}Ga
6	J.B.	Hodgkin's disease										−	−			None	None	Similar
7	D.K.	Hodgkin's disease	Stage III (left neck, both hilar regions and para-aortic nodes)	±	+			−	+			−	+			Poor	Good	^{67}Ga
8	J.D.	Hodgkin's disease	Stage IIIA (right neck, hilum, and spleen) post-laparotomy	+	+			+	−							Fair	Good	^{67}Ga
9	H.F.	Carcinoma bronchus	Thin-walled, necrotic, 3 cm lesion			−	−									None	None	Similar
10	D.T.	Carcinoma bronchus	Rapidly enlarging lesion—no histology			±	+									Fair	Good	^{67}Ga
11	H.H.	Carcinoma of bronchus	1 cm lesion. Negative chest X ray			−	−									None	None	Similar
12	W.H.	Carcinoma bronchus	Recurrent tumour			±	+									Fair	Good	^{67}Ga
13	F.M.	Seminoma testis	Mediastinal deposit	±	±											Fair	Fair	Similar
14	L.W.	Seminoma testis	Recurrent deposit right lung			−	+									None	Good	^{67}Ga

No.	Name	Disease	Clinical features and treatment	Relative uptake of ^{111}In-bleo and ^{67}Ga												Clinical correlation		Best tumour-imaging agent
				Medfast.		Lung		Neck		Axilla		Abdo.		Other				
				In	Ga	In	Ga	In	Ga	In	Ga	In	Ga	In	Ga	In	Ga	
16	J.M.	Lymphoma (histiocytic)	Neck, axillary and inguinal glands					−	−	−	−	−	−			None	None	Similar
17	R.M.	Osteosarcoma	Localized to sternum	+	+											Good	Good	Similar
18	J.M.	Myeloma	Soft tissue mass left chest											+	+	Good	Good	Similar
19	Z.J.	Hydrocoele testis	A small hydrocoele							+	+					Good	Good	Similar
20	T.G.	Hodgkin's disease	Stage IA (Left groin)											−	−	None	None	Similar

Table 2.1 (cont.)

± Denotes equivocal positive uptake.

TABLE 2.11

PATIENTS RECEIVING TREATMENT BETWEEN SCANS

No.	Name	Disease	Clinical features and treatment	Relative uptake of ^{111}In-bleo and ^{67}Ga												Clinical Correlation		Best tumour-imaging agent
				Mediast.		Lung		Neck		Axilla		Abdo.		Other				
				In	Ga	In	Ga	In	Ga	In	Ga	In	Ga	In	Ga	In	Ga	
21	S.G.	Hodgkin's disease	Infiltration of right upper zone of lung. Chemotherapy after Ga and one week before In			−	−									None	None	Similar
22	E.R.	Hodgkin's disease	Diffuse lung nodules, root of neck. Chemotherapy started after In, four days before Ga			−	−	±	+							Poor	Fair	^{67}Ga
23	R.M.	Lymphoma	Mediastinal and para-aortic nodes. DXR to para-aortic nodes before In	+	+							−	+			Good	Good	Similar
24	M.F.	Teratoma testis	Multiple lung deposits. Chemotherapy before Ga and after In			−	−									None	None	Similar
25	F.M.	Seminoma testis	Paravertebral intrathoracic deposits. Chemotherapy before Ga and after In			−	−									None	None	Similar
26	R.M.	Osteo-sarcoma	Primary in sternum with neck node involvement. DXR to left neck before Ga					±	+					+	+	Fair	Good	^{67}Ga
27	M.D.	Melanoma	Deposits in axilla and infra-clavicular nodes. Chemotherapy ,six weeks before In and eight weeks before Ga							±	+			±	+	Fair	Good	^{67}Ga
28	M.C.	Ca ovary	Pelvic mass and pleural effusion. Chemotherapy before Ga and after In	−	−							−	−			None	None	Similar

in 14 examinations, the scans were similiar. Clinical correlations are given in Table 2.3.

The photographs of the scans illustrate typical grading assessments of the clarity of tumour image. They show quite clearly the superiority of ^{67}Ga-citrate as a tumour imaging agent in these clinical situations (Figures 2.1 - 2.6.).

The concentrations of ^{67}Ga and ^{111}In found in 17 samples of spleen removed 24 hours after simultaneous administration of ^{67}Ga-citrate and ^{111}In-bleomycin are listed in Table 2.4. The data are listed as the percentage of the administered dose per kilogram wet tissue ± one Standard Deviation, between four and ten individual samples of each spleen were assayed. Seven of the 17 spleens examined showed macroscopic or histological evidence of infiltration by tumour.

Similar data for nine samples of liver and six samples of lymph nodes are also present in Table 2.5.

Concentrations of ^{67}Ga and ^{111}In were compared in whole blood and plasma samples during the first 24 hours after administration in 18 patients. IN 13 patients the rate of clearance of ^{111}In from the blood was slower than that of ^{67}Ga, a typical set of clearance curves is illustrated in Fig. 27A. Four patients showed a more rapid clearance of ^{111}In than that of ^{67}Ga. In two patients the clearance of ^{111}In was exceptionally slow; the loss from the blood between one hour and 70 or 120 hours approximating in each case to a exponential with half-times of 85 hours and 70 hours. One of these cases is illustrated in Fig. 27B. The tissue to blood concentration ratios showed wide variations and the mean values and the ranges for

TABLE 2.111

	^{67}Ga-citrate	^{111}In-bleomycin
GOOD	15	4
FAIR	3	9
POOR	1	3
NONE	9	12

Correlation of ^{67}Ga-citrate and ^{111}In-bleomycin scans to ultimate clinical and pathological findings.

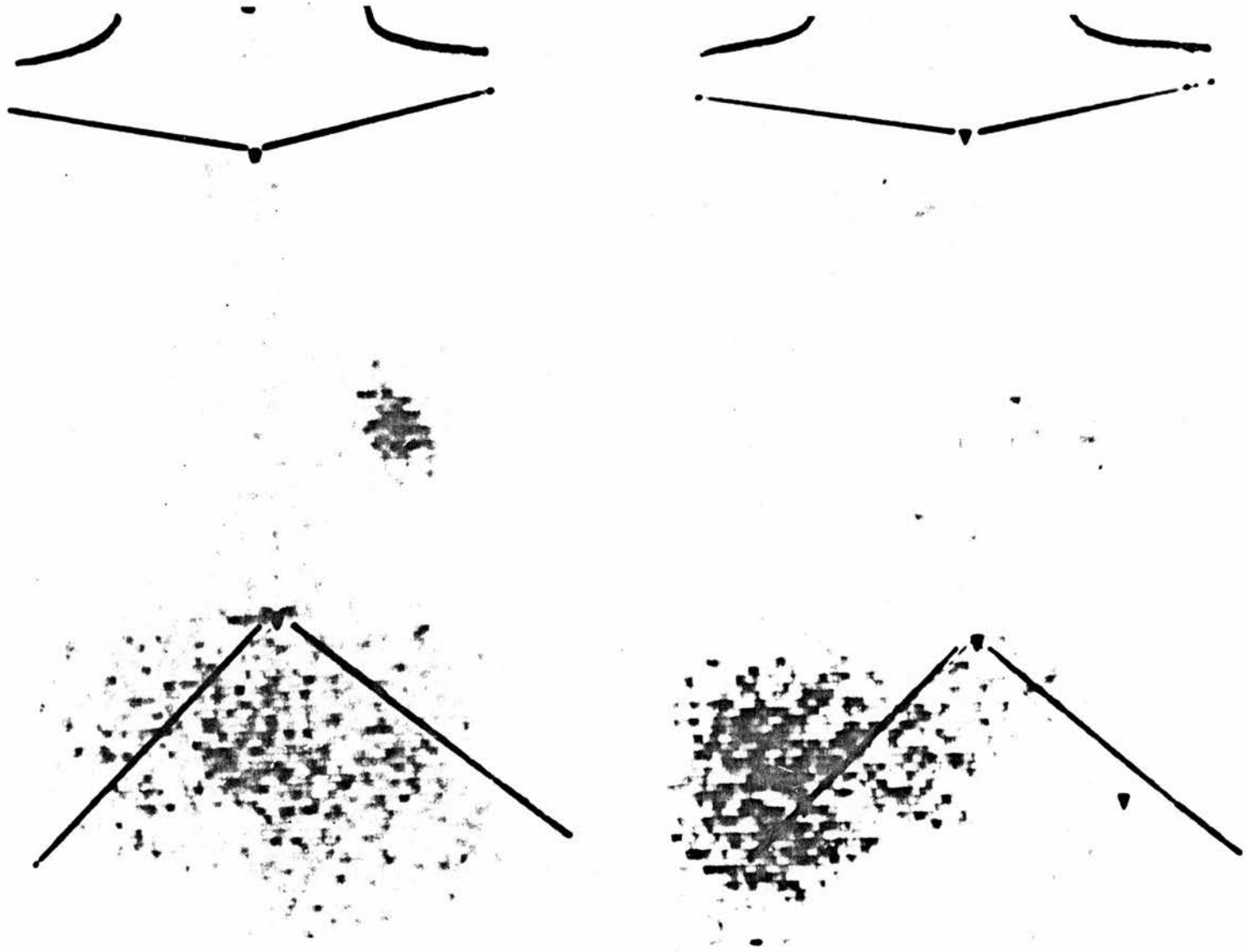


FIG. 2.1 and 2

Figure 2 shows the scan of chest and upper abdomen using ^{67}Ga-citrate in a patient with a carcinoma of the bronchus.

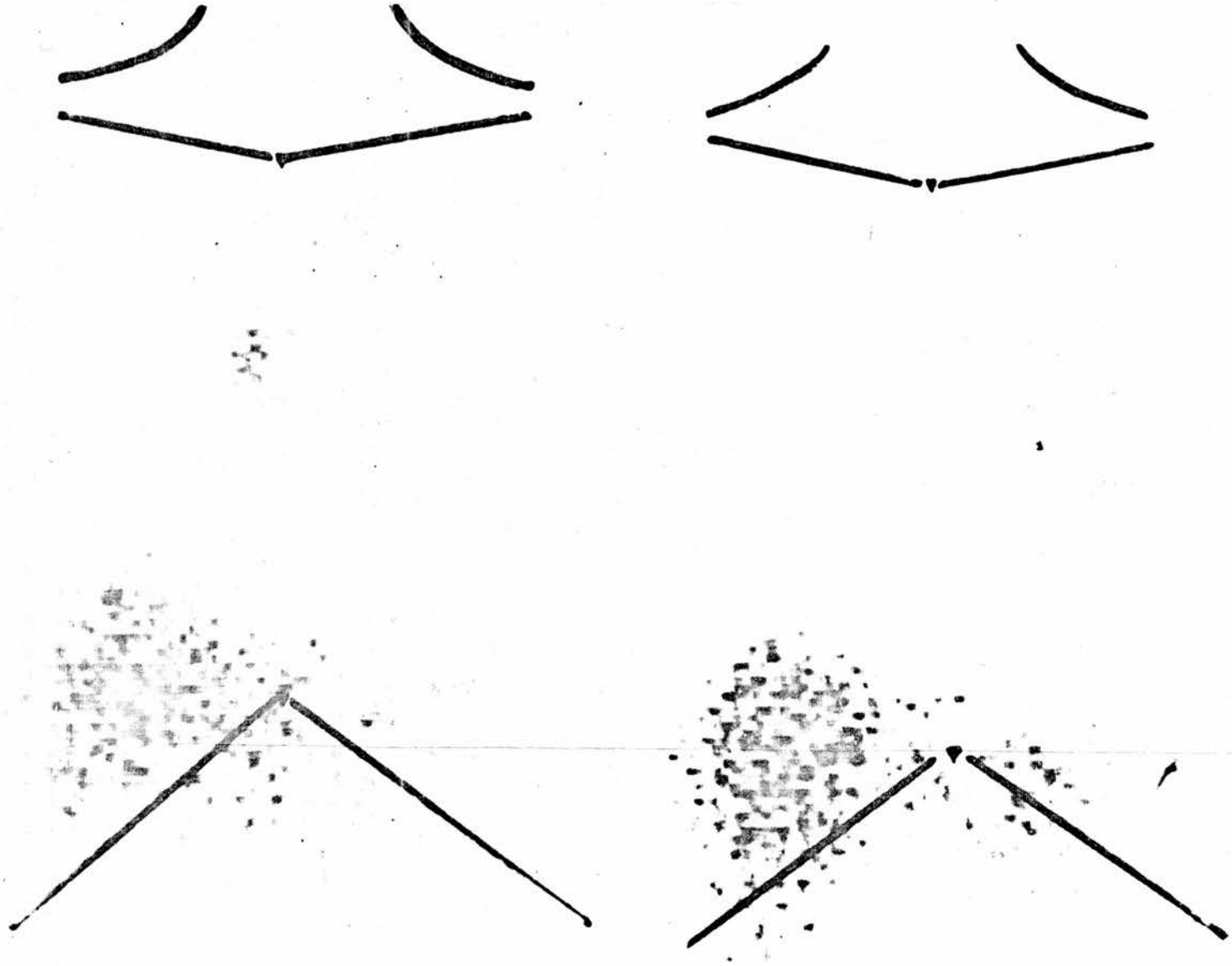


FIG. 2, 3 and 4

Figure 4 shows a chest scan using ^{67}Ga-citrate of a patient with Hodgkin's disease. The right hilar node is much more clearly seen than in the ^{111}In-bleomycin scan (Fig. 5).

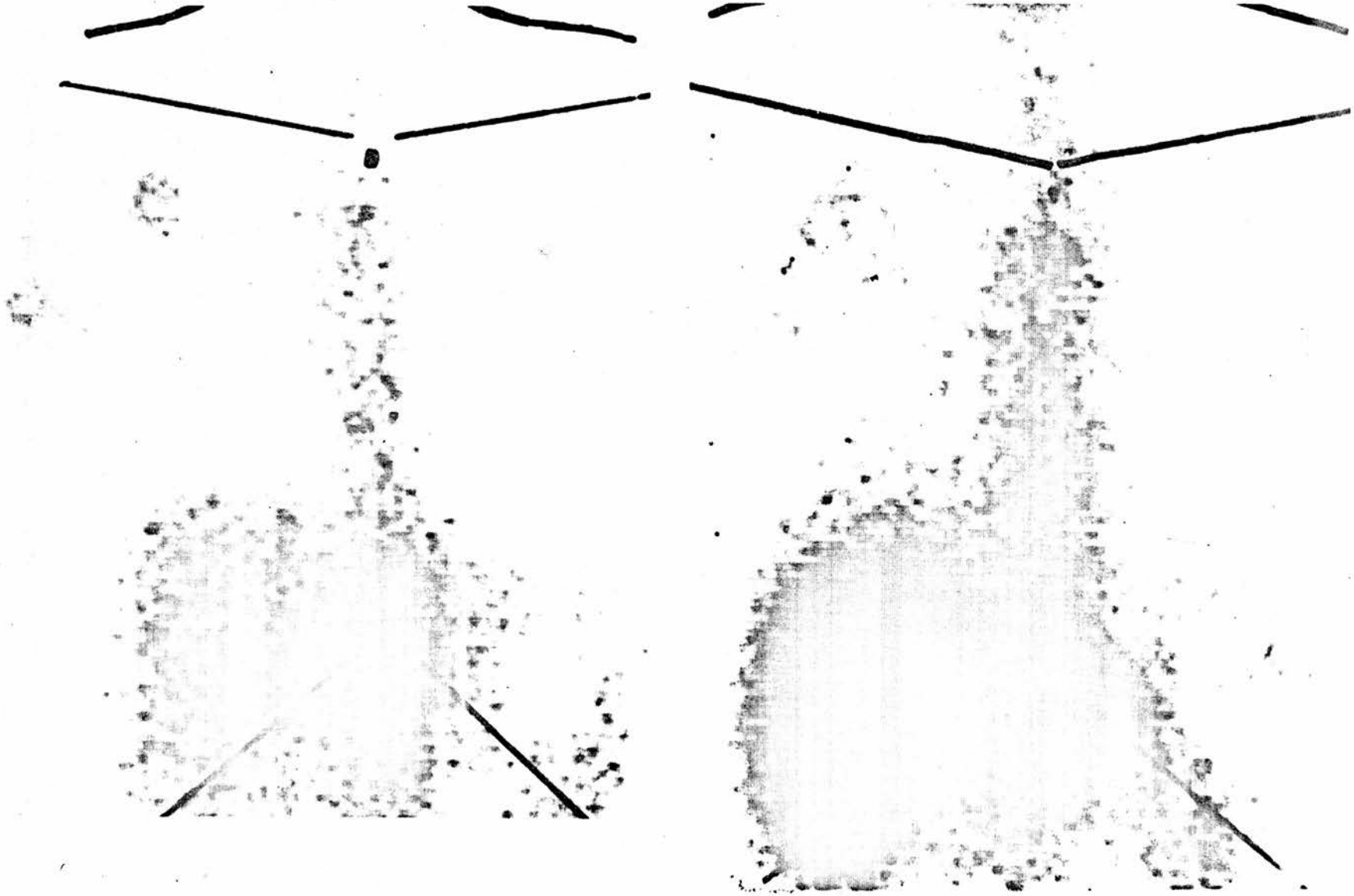


FIG. 2.5 and 6

Figure 6 again shows a chest scan using ^{67}Ga-citrate of a patient with malignant melanoma with deposits in the axillary and infraclavicular nodes. The ^{111}In-bleomycin scan (Fig. 7.), while demonstrating some uptake, show less accurate localization than the ^{67}Ga-citrate scan.

TABLE IV

COMPARISON OF THE CONCENTRATIONS OF ^{67}Ga AND ^{111}In (% DOSE/KG WET TISSUE) IN SPLEEN, LIVER AND LYMPH NODES 24 HOURS AFTER ADMINISTRATION OF ^{67}Ga CITRATE PLUS ^{111}In-BLEOMYCIN

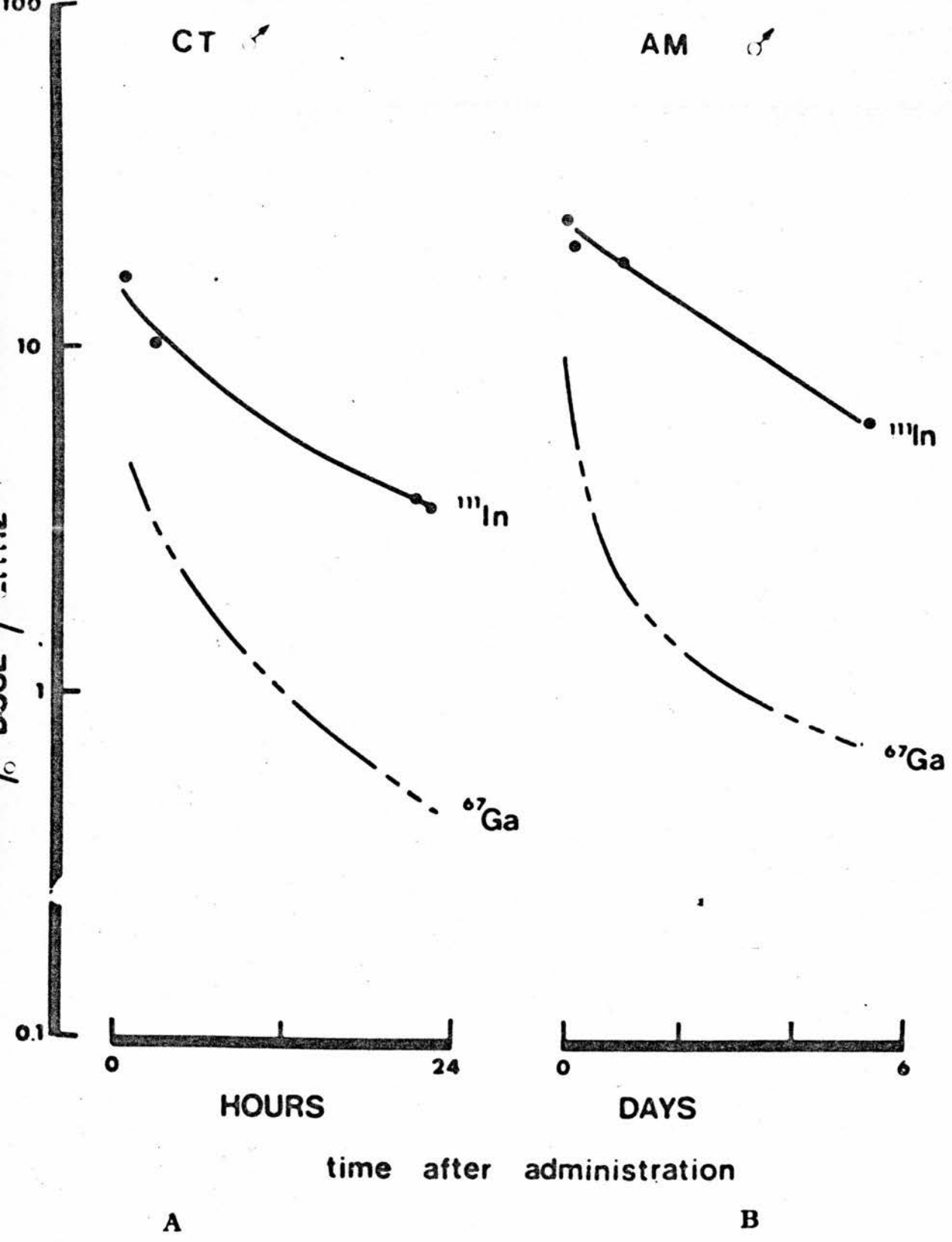
Patient	Age and sex	Diagnosis	Spleen			Liver			Lymph nodes		
			^{67}Ga	^{111}In	$^{67}Ga/^{111}In$	^{67}Ga	^{111}In	$^{67}Ga/^{111}In$	^{67}Ga	^{111}In	$^{67}Ga/^{111}In$
T.K.	29 m	HD – MC	4·9 ± 0·2†	7·2 ± 0·6	0·68*	9·0	12·8	0·71			
W.Wo.	23 m	HD – MC (IA)	6·1 ± 5·4	2·2 ± 1·0	2·76	10·0	4·7	2·11			
F.Ho.	26 m	HD – NS	5·7 ± 0·9	11·9 ± 1·8	0·48*						
A.M.	59 m	HD – NS	3·5 ± 0·1	12·6 ± 0·6	0·28	8·2	29·5	0·28			
P.T.	52 f	HD – MC (IIA)	6·1 ± 0·1	4·1 ± 0·1	1·48	5·5	7·6	0·72	39·8	4·3	9·43*
A.Wh.	31 m	HD – NS	8·7 ± 1·3	6·5 ± 1·0	1·35	7·8	5·8	1·36			
R.D.	26 m	HD – LP (IA)	6·8 ± 1·1	5·8 ± 1·0	1·18	17·2	14·0	1·23			
A.We.	45 m	HD – LD	2·9 ± 0·1	4·6 ± 0·1	0·62	5·5	8·3	0·66			
C.Th.	22 m	HD – MC	6·5 ± 0·1	6·0 ± 0·1	1·08*	10·2	11·3	0·90	13·4	11·3	1·19*
R.H.	38 m	HD – LD	2·9 ± 0·1	6·2 ± 0·1	0·47*	2·7	7·9	0·34	8·5	6·4	1·39*
G.O'H.	24 m	HD – MC	6·7 ± 0·6	7·9 ± 0·5	0·85*						
K.G.	29 m	HD – NS	4·0 ± 0·2	0·9 ± 0·0	4·24						
P.Wa.	24 m	HD – MC	6·6 ± 0·1	9·1 ± 0·3	0·72*				15·7	21·8	0·72*
E.C.	47 f	Lymphocytic lymphoma	4·7 ± 0·1	1·1 ± 0·1	4·21				2·9	1·0	2·90
R.L.	25 m	Hereditary spherocytosis	3·2 ± 0·2	0·7 ± 0·1	4·34						
A.Lo.	57 f	Follicular lymphoma	1·5 ± 0·1	4·1 ± 0·1	0·37				1·1	1·8	0·61
L.K.	19 m	HD – MC	7·2 ± 0·5	1·1 ± 0·18	6·77*				15·3	2·2	6·95*

HD—Hodgkin's disease (MC—mixed cellularity; NS—nodularsclerotic; LP—lymphocyte predominant; LD—lymphocyte epleted).
—Tumour infiltrated tissue.
—Standard deviation.

TABLE 2.5

Tissue to blood ratios in spleen, liver and lymph nodes 24 hours after ^{67}Ga citrate and ^{111}In-bleomycin.

	^{67}Ga		^{111}In	
	Mean	Range	Mean	Range
Spleen	3.0	1.5 to 13.8	1.3	0.2 to 5.4
Liver	5.4	1.9 to 13.8	2.2	0.5 to 4.8
Lymph nodes	14.2	0.8 to 35.8	1.8	0.6 to 3.4



ig 2.7

he disappearance of ^{67}Ga and ^{111}In from the blood following simultaneous administration of ^{67}Ga-citrate and ^{111}In-eomycin. Patient C.T. illustrates the clearance pattern bserved in the majority of the patients studied. Patient .M. illustrates the abnormally slow clearance by ^{111}In observed in two patients.

spleen, liver and lymph nodes are listed in Table 2.5.

DISCUSSION

It is essential that the clinical assessment of tumour-imaging agents in the future should be by controlled trial. The initial enthusiasm for a new diagnostic agent is understandable, but little knowledge can be gained from the publication of scanning results using a single agent other than that the agent is suitable for assessment. It is known that certain tumour-imaging agents, for example ^{75}Se-selenomethionine, ^{197}Hg-chlormerodrin, ^{67}Ga-citrate and ^{111}In-bleomycin, can all demonstrate a proportion of malignant lesions in a range of tumours; what we do need to know is whether a particular agent demonstrates particular groups of tumours or whether one tumour-imaging agent gives higher tumour-background radioactivity ratios than another.

In this study, it has been shown that ^{67}Ga-citrate is a more useful tumour-imaging agent than ^{111}In-bleomycin both in clinical correlation and in the quality of image obtained for lesions situated above the diaphragm. It is difficult to evaluate the place of ^{111}In-bleomycin in the detection of abdominal tumours because insufficient numbers of infradiaphragmatic lesions have been scanned from Hodgkin's disease. ^{111}In-bleomycin's favourable property of renal excretion together with the known affinity of bleomycin for serous membranes may make it a useful agent for the

detection of gut and pelvic tumours. Uptake into faeces and small intestine is a well-known drawback to the use of ^{67}Ga-citrate in the demonstration of such tumours.

There have been a number of trials investigating ^{111}In-bleomycin as a tumour imaging agent. Lilien et al (1974) have reported very high scanning success rates in neoplasms normally difficult to detect by scanning techniques. They had successful scans in all of 14 patients with malignant melanoma, but their most remarkable figures were 88 per cent positive scans in 15 patients with ovarian carcinomas. Using ^{67}Ga-citrate, Symmonds and Tauxe (1972) had disappointing experiences in gynaecological tumours, particularly squamous cell carcinomas of the cervix. Clearly the scanning of gynaecological malignancies with ^{111}In-bleomycin needs full appraisal, although in our one patient with a large ovarian carcinoma, neither the ^{111}In-bleomycin nor the ^{67}Ga-citrate scans showed the tumour. The same group (Jones et al., 1973) earlier reported their work in lymphomas, finding a 79% overall accuracy in the prediction of disease sites, which is similiar to ^{67}Ga-citrate studies in these conditions (Kay and McCready, 1972; Turner et al., 1972). Goodwin et al (1974) reported a 69% scanning success rate in a variety of tumours and Verma et al. (1973) reported an overall success rate of 79%.

However, diagnostic success rates using tumour imaging agents depend not only on the type of tumour but also on the area

and extent of disease. When only one agent is used, the results can be misleading in the assessment of that agent, and a study comparing two or even more agents, is more helpful in the assessment of that agent. This suggestion is supported by a recently published trial by Grove et al., (1974), who compared scans using ^{111}In-bleomycin, ^{67}Ga-citrate and ^{57}Co-bleomycin. The ^{57}Co-bleomycin seemed to be better than those obtained with ^{67}Ga-citrate and clearly one of the next steps is to investigate ^{57}Co-bleomycin more thoroughly and decide whether the possible advantages of ^{57}Co-bleomycin outweigh the disadvantage of its long physical half-life.

The mechanism of uptake of ^{111}In-bleomycin into tumours, like other tumour-imaging agents, is not clear. It has been suggested that it is the bleomycin which increases the concentration of ^{111}In-bleomycin by its own concentration within tumours. There is, in fact, some evidence that concentration of bleomycin actually occurs in certain mouse tumours. It is not clear, however, whether ^{111}In actually enters the tumour as ^{111}In-bleomycin. It is likely that the improvement in tumour:background ratio of ^{111}In-bleomycin over ^{111}In-transferrin is due to the rapid renal excretion of ^{111}In-bleomycin thereby rapidly lowering background activity. If the chelate is not completely stable, as it would appear not to be (Goodwin et al., 1974), ^{111}In-transferrin will be formed to some extent and background activity will be heightened.

At a cellular level, the reason why ^{111}In-bleomycin or ^{111}In-transferrin enters tumours remains unknown.

In the second part of this study the uptakes of ^{67}Ga-citrate and ^{111}In-bleomycin in involved and normal lymph nodes, spleen and liver, removed at operation were measured. In addition, the blood clearance of the two agents was measured over the twenty-four hours prior to operation. Broadly, these results, although restricted to lymphomas, have tended to support the clinical observations, although one or two exceptions have been noted.

Comparison of the data presented in Table 2.4 shows that in the spleen, liver and lymph nodes there are wide variations in uptake both between the two nuclides and between different tissues. In the spleen, nine of the 17 samples contained more ^{67}Ga than ^{111}In and eight contained more ^{111}In than ^{67}Ga; the ratios of the highest and lowest concentrations observed were 6:1 for each nuclide. Only two out of seven samples of lymph nodes contained more ^{111}In than ^{67}Ga but this tissue showed the greatest range in the concentrations of the two nuclides in individual samples, the highest to lowest concentration ratios being 36:1 for ^{67}Ga and 22:1 for ^{111}In. The concentrations of ^{67}Ga observed in spleen and liver were similiar to those previously reported by Saunders, Taylor and Trott (1973).

Seven of the spleen samples were infiltrated with tumour and five of these contained less ^{67}Ga than ^{111}In; in contrast, four of

the five tumour-infiltrated lymph nodes showed ^{67}Ga concentrations greater than those of ^{111}In. None of these variations could be simply correlated with the type or stage of the disease.

The rate of clearance of the two nuclides from the blood showed considerable variation and the tissue to blood concentration ratios varied widely as is seen in Table 2.5. The rates of clearance of ^{67}Ga from the whole blood and from the plasma were similiar to those reported in earlier studies (Saunders et al., 1973). The clearance of ^{111}In from both the whole blood and plasma showed greater variation than that of ^{67}Ga. In most cases the clearance of ^{111}In from the blood was slower than that of ^{67}Ga and two cases showed an unusually slow clearance of ^{111}In. The majority of the cases did not show the rapid initial clearance of ^{111}In from the blood with half-times of about 15 minutes which have been reported by other workers (Williams, Merrick and Lavender, 1974: Goodwin et al., 1973). The reasons for this slower clearance are uncertain but it may possibly reflect the fact that most of the cases were patients in whom the tumour mass was small. In another study the clearance of ^{111}In-bleomycin from the blood was studied in an 18-year-old man suffering from Hodgkin's disease and with a large mass in the mediastinum. At the commencement of treatment, with radio- and chemotherapy, 97% of the ^{111}In disappeared from the blood in 24 hours, but two, four, and nine weeks later when the mediastinal mass had almost entirely disappeared, the rate of blood clearance had slowed and only 90% of the dose disappeared during the

first 24 hours after an injection of ^{111}In-bleomycin. Thus it seems possible that the presence of large tumour masses may result in a more rapid clearance of ^{111}In from the blood after injection of the bleomycin complex.

The results of these studies of the tissue uptake of ^{67}Ga and ^{111}In, after administration as the citrate and bleomycin complexes respectively reinforce the conclusion drawn from the scanning studies that ^{111}In-bleomycin offers no advantages over ^{67}Ga-citrate for the investigation of lymphomas (including Hodgkin's disease) and other tumours situated above the diaphragm.

2b: A CLINICAL COMPARATIVE STUDY OF THE TUMOUR IMAGING AGENTS ^{67}GALLIUM-CITRATE AND ^{57}CO-BLEOMYCIN

A summary of this section has been submitted as a letter to the British Journal of Radiology (to be published)

INTRODUCTION

Reports from France (Nouel et al 1973) and from the United States (Grove et al 1975) have suggested that bleomycin labelled with ^{57}Co is a useful tumour imaging agent. The rationale for the use of labelled bleomycin has been examined in a previous section. ^{57}Co forms a stable chelate with the polypeptide bleomycin and, unlike ^{111}In, localization of ^{57}Co in the bone marrow is not a problem. The main disadvantage of using a radionuclide such as ^{57}Co is its long physical half-life (270 days) which gives rise to problems of waste disposal.

METHODS

The patients with their informed consent, received 1 mCi of ^{57}Co labelled bleomycin intravenously, blood samples being taken at 1, 3, and 24 hours and urine being collected for 24 hours after the injection. Scanning was carried out at 6 hours and 24 hours after the injection. Approximately one week later, the same patients received 2.5 mCi of ^{67}Ga-citrate and were scanned 48 hours later.

For the laparotomy studies, 100 mCi of ^{57}Co-bleomycin and 250 mCi ^{67}Ga-citrate were injected intravenously 24 hours prior to operation. Blood samples were taken at 1, 3, and 24 hours after the injection. Biopsy samples were cut into five or more pieces

weighed and assayed for ^{57}Co and ^{67}Ga. Standards of the injected agents were also assayed.

The following settings were used:

^{57}Co Peak --- Channel 1.

0.5 MeV upper 6.0 - lower 5.0 --- Channel 2.

Using these settings there was no ^{57}Co spillage into the ^{67}Ga channel, but ^{67}Ga gave approximately 1.2 times the amount in the ^{57}Co channel as in the ^{67}Ga channel.

RESULTS:

Nine patients with a variety of tumours were scanned with ^{57}Co-bleomycin and also received a control scan with ^{67}Ga-citrate. Two patients did not receive ^{67}Ga-citrate control scans. Two patients had large inoperable carcinomas of the pancreas which failed to image either with ^{57}Co-bleomycin or ^{67}Ga-citrate. In 2 patients, one with a carcinoma of the bronchus and one with Hodgkin's disease the ^{67}Ga-citrate scan was unequivocably superior to the ^{57}Co-bleomycin. In one patient with a malignant pleural effusion, the ^{57}Co-bleomycin scan was superior in that there was a diffusely increased area of uptake in the region of the pleural effusion whereas the ^{67}Ga-citrate scan was negative.

The results of the biopsy specimens taken at laparotomy for the staging of Hodgkin's disease after receiving small doses of ^{57}Co-bleomycin and ^{67}Ga-citrate are given in Table 2.8. In all, seven patients were studied. As can be seen from the Table, the tissue:plasma ratios of the biopsy samples were usually higher for ^{57}Co than for ^{67}Ga, although the absolute amounts of radionuclide detected were considerably greater for ^{67}Ga than for ^{57}Co. The significance of this will be discussed in the next section.

DISCUSSION:

The scanning results with ^{57}Co-bleomycin were disappointing.

PATIENT	ISOTOPE	PLASMA % DOSE/L			SPLEEN % DOSE/Kg	LYMPH NODES % DOSE/Kg				
		1 HR.	3 HR.	24 HR.		PARA-AORTIC	PORTA HEP.	ILIAC	JEJ.	SPLENIC
R.H.	^{67}Ga	15.10	10.56	4.02	3.19± 0.11		.93	3.94	2.78	
	^{57}Co	5.52	3.05	0.93	1.46± 0.2		2.31	3.28	1.19	
C.M.	^{67}Ga			2.81	4.04± 0.15	1.59				8.26
	^{57}Co			0.06	0.82± 0.46	0.83				0
J.S.	^{67}Ga	18.9	15.18	4.35		4.99		2.62		
	^{57}Co	2.49	2.26	*		0.7		0.4		
P.A.	^{67}Ga	15.08	9.51	4.34		2.04				
	^{57}Co	3.33	0.5	*		0.91				
R.N.	^{67}Ga	10.36	6.98	1.97	1.91± 0.41	3.77	7.28		0.00	
	^{57}Co	5.1	2.29	0.2	0.47± 0.14	0.30	0.06		0.00	
G.K.	^{67}Ga	21.44	14.24	4.92	5.89	12.07				
	^{57}Co	6.15	2.83	0.24	0.17	1.96				
S.W.	^{67}Ga	10.6	3.61	1.18	21.13± 2.18	3.21				
	^{57}Co	5.38	1.61	0.34	7.08± 4.61	0.84				

* The plasma activity of ^{57}Co was below the limits of detection

The low amounts of ^{57}Co detectable led to extremely low information densities which meant unacceptably long scanning times in a busy department dealing with ill patients. It is interesting that the biopsy specimens of spleen and lymph nodes which were involved with Hodgkin's diease showed higher tissue:plasma ratios for ^{57}Co than for ^{67}Ga. However, the absolute amounts of ^{57}Co present were often so low that using currently available instrumentation, scanning would have presented a major problem due to low information densities.

Clinical studies using ^{57}Co-bleomycin have been reported by Mamo et al (1973), Suzuki et al (1974), and Grove et al (1974). In the study by Mamo et al (1973), ^{57}Co-bleomycin was found to be superior to $^{99}Tc^{m}$ for the detection of intracranial metastases although for meningiomas and gliomas, $^{99}Tc^{m}$ appeared to be superior. Grove et al (1974) compared ^{57}Co-bleomycin scans with ^{67}Ga-citrate in 15 patients, finding that a slight superiority in imaging was obtained with ^{57}Co-bleomycin.

In view of our results which appeared to be at variance with those of other centres, chromatography was performed on blood and urine specimens and standard distributions were obtained showing that no dissociation of the injected material had occured.

Although the scanning results in this series were disappointing, the biopsy results do suggest that ^{57}Co can localize in tumours in higher relative concentrations than ^{67}Ga, although the absolute amounts of ^{57}Co in the body is so small that scanning is extremely difficult and time consuming. It may be that certain tumour types

concentrate ^{57}Co preferentially as suggested by Mamo et al (1973). However, in our hands, the possible advantages of ^{57}Co-bleomycin did not outweigh the disadvantages associated with the use of this agent on a large scale.

3. THE CLINICAL SIGNIFICANCE OF ^{67}Gallium Uptake

1. Hodgkin's Disease

A modified version of this section has been submitted for publication to the European Journal of Nuclear Medicine.

INTRODUCTION

^{67}Gallium-citrate (^{67}Ga-citrate) whole-body scanning has been used in the management of Hodgkin's disease for several years. Currently ^{67}Ga-citrate scanning is used for initial staging, and in cases of suspected relapse. Most of the reports on the use of ^{67}Ga-citrate scanning in Hodgkin's disease have concentrated on its place in clinical management. This paper attempts to relate ^{67}Ga uptake to the disease process. It was stimulated by the clinical impression that high uptakes in disease areas were seen in ill patients, patients in relapse or patients with widespread disease. On the other hand, there are occasional cases where large tumours in areas such as the mediastinum show little or no concentration of ^{67}Ga-citrate. A retrospective study of ^{67}Ga-citrate scans has been performed in patients scanned over the last five years at the Royal Marsden Hospital, Sutton, in an attempt to correlate various clinical parameters with the level of ^{67}Ga radioactivity.

METHODS

The patients in this study were either previously untreated patients, or patients who had relapsed following treatment.

Between 2-3 mCi of ^{67}Ga-citrate (commercially obtained from Philips-Duphar) was injected intravenously. Scanning was carried out 48 hours later on a Picker Magnascanner or a Selo Superscanner D.S.7. The patients received laxatives routinely, but if a doubtful area was seen on the abdominal scan, the examination was repeated 24 hours later after further laxatives. The scans were reported by two independent observers. On some occasions whole body scanning was not routinely performed, scans being limited to the neck and chest. If an abdominal scan was not performed in a patient with confirmed disease in the abdomen, the patient was excluded from the study even if the disease was present above the diaphragm. This has possibly led to a slightly higher proportion of lesions above the diaphragm being studied than would have been the case had whole-body scanning been routinely performed.

Tumour concentration of ^{67}Ga seen on scanning was qualitatively assessed on a four level scale, using the radioactivity level of the liver as a reference point. Thus tumour uptake was:-

0.	Not imaged.
L. (Low)	Imaged, but only minimally, the level of ^{67}Ga uptake being less than that of the liver.
M. (Moderate)	Imaged, the level of ^{67}Ga uptake being moderate and equivalent to that of the liver.

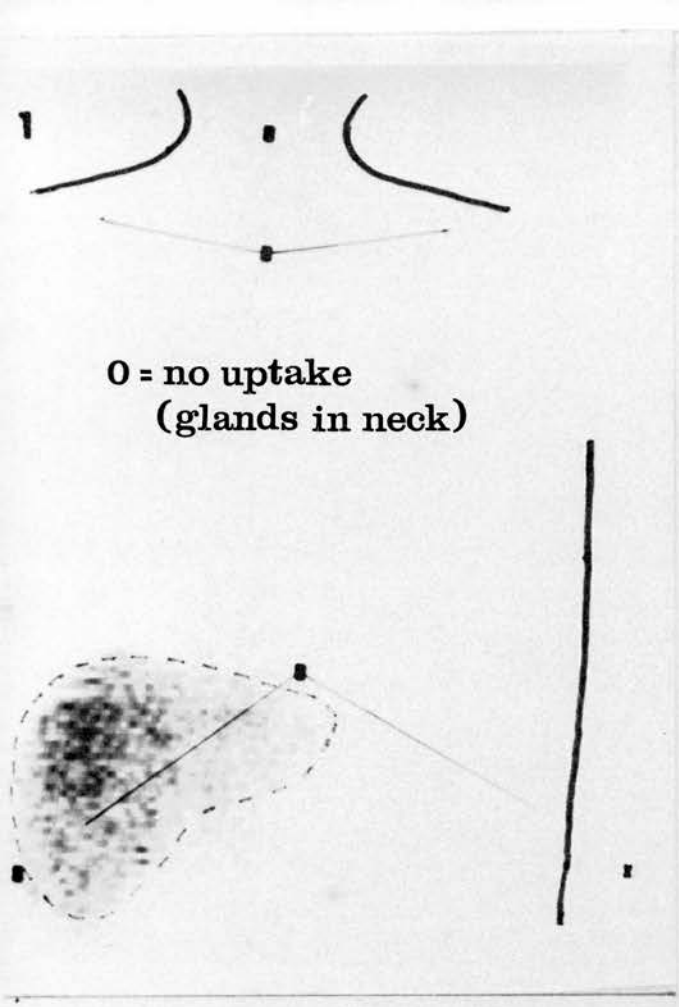
H (High) Imaged, and the level of ^{67}Ga uptake was high being greater than that of the liver. (See Figure 3.1).

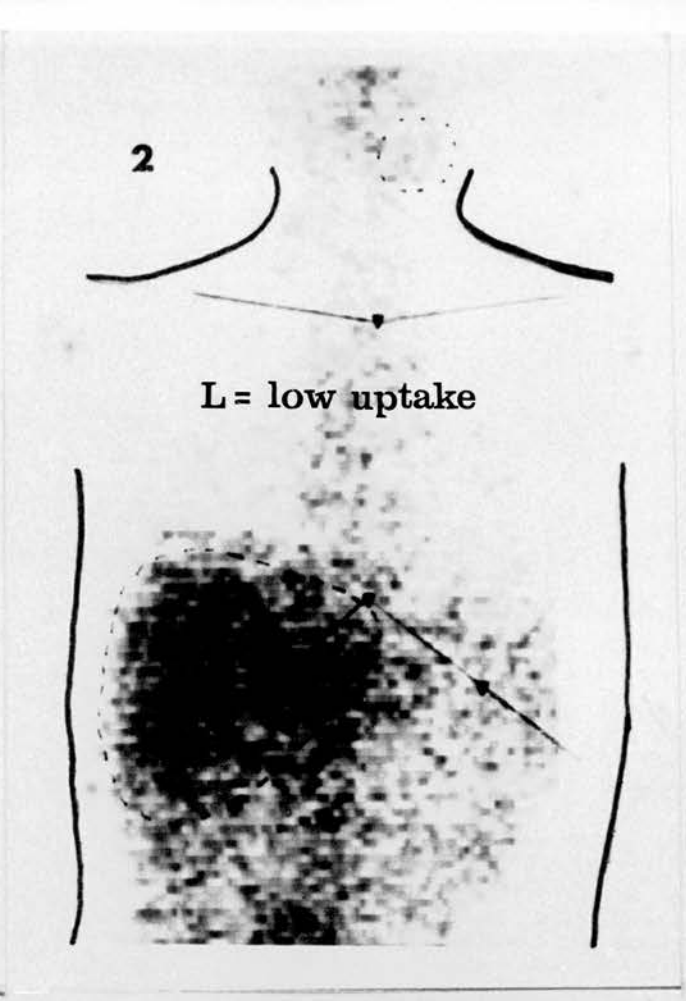
We found this classification satisfactory, virtually all the lesions falling easily into one of the categories. The category of each lesion was then correlated with its histology and anatomical site. The histology was reviewed by the Department of Pathology, Royal Marsden Hospital, (Professor N. Gowing) and reported according to the Rye modification of the classification of Lukes and Butler (1966).

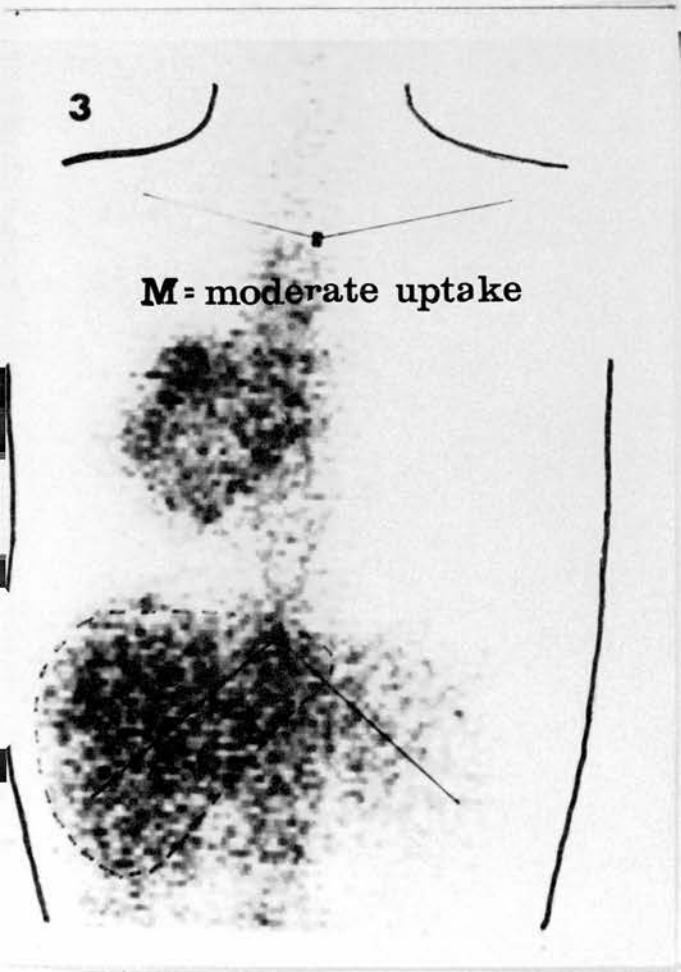
Patients were categorized according to the level of ^{67}Ga uptake and this was correlated with the stage of the disease, remission length and survival.

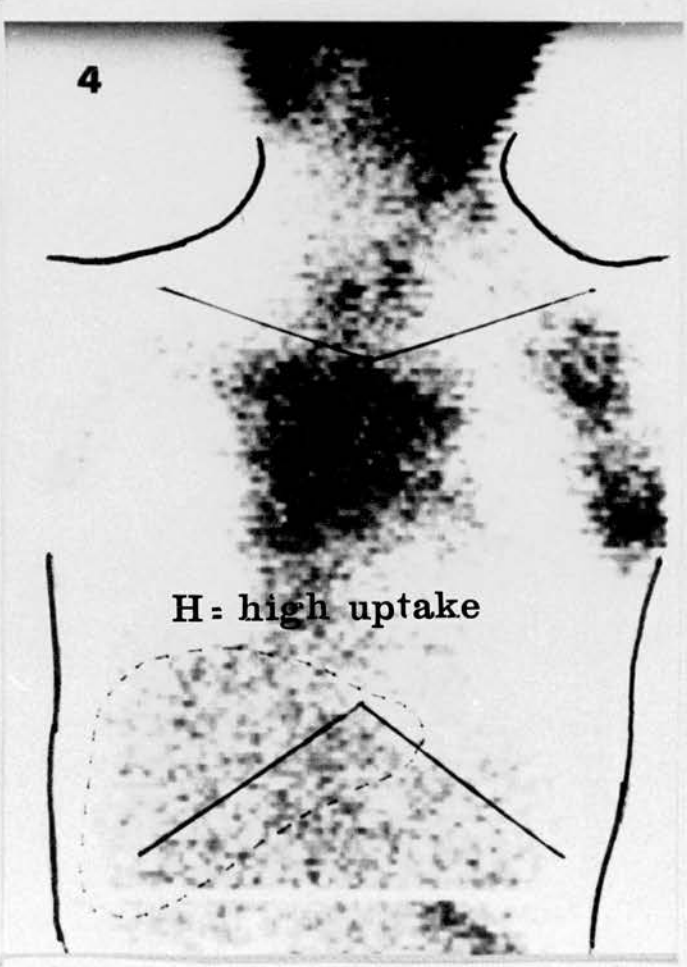
The presence of disease was determined by clinical examination, radiology (including lymphography), isotope and ultrasound techniques, pathology, biopsy and, in many cases, laparotomy.

The patients were staged according to the system introduced at Ann Arbor (Carbone et al 1972). Patients who relapsed were re-staged. The length of remission following treatment was then determined. There were two groups of patients. The first group consisted of previously untreated patients with Hodgkin's disease who were scanned on presentation. The second group consisted of patients who had received treatment and who were scanned on relapse. If a patient had more than one involved area, and these areas had different levels of ^{67}Ga uptake, then the patient was


1
0 = no uptake
(glands in neck)


2
L = low uptake


3
M = moderate uptake


4
H = high uptake

categorised according to the lesion showing the maximum uptake.

RESULTS

In all, 89 patients with Hodgkin's disease were studied. These patients had a total of 241 anatomical sites involved with Hodgkin's disease, giving an overall percentage of positive scans of 62%. This percentage of positive scans is lower than most other reported series, and is due to our method of subdivision of anatomical sites. Most series have grouped right and left lymphatic groups together, for example, neck, axillae, and supraclavicular regions. It was decided to separate these into right and left regions since both sides of a region may be involved with Hodgkin's disease, but the scan may only show uptake on one side. Separating the regions lowers the scanning success rate when compared with other series where regions rather than sites were examined.

Table 3.1 shows the ^{67}Ga uptake characteristics for all the diseased areas grouped into the four histological sub-classifications. Only a very small percentage of lesions of "lymphocyte predominant" histology concentrated ^{67}Ga sufficiently to be imaged. There is a clear improvement in the ability to image the "nodular sclerosis", "mixed cellularity", and "lymphocyte depleted" groups, but the number of patients in the "lymphocyte predominant" group is small and the apparent difference in imaging properties, although suggestive, is not statistically significant.

LE 1

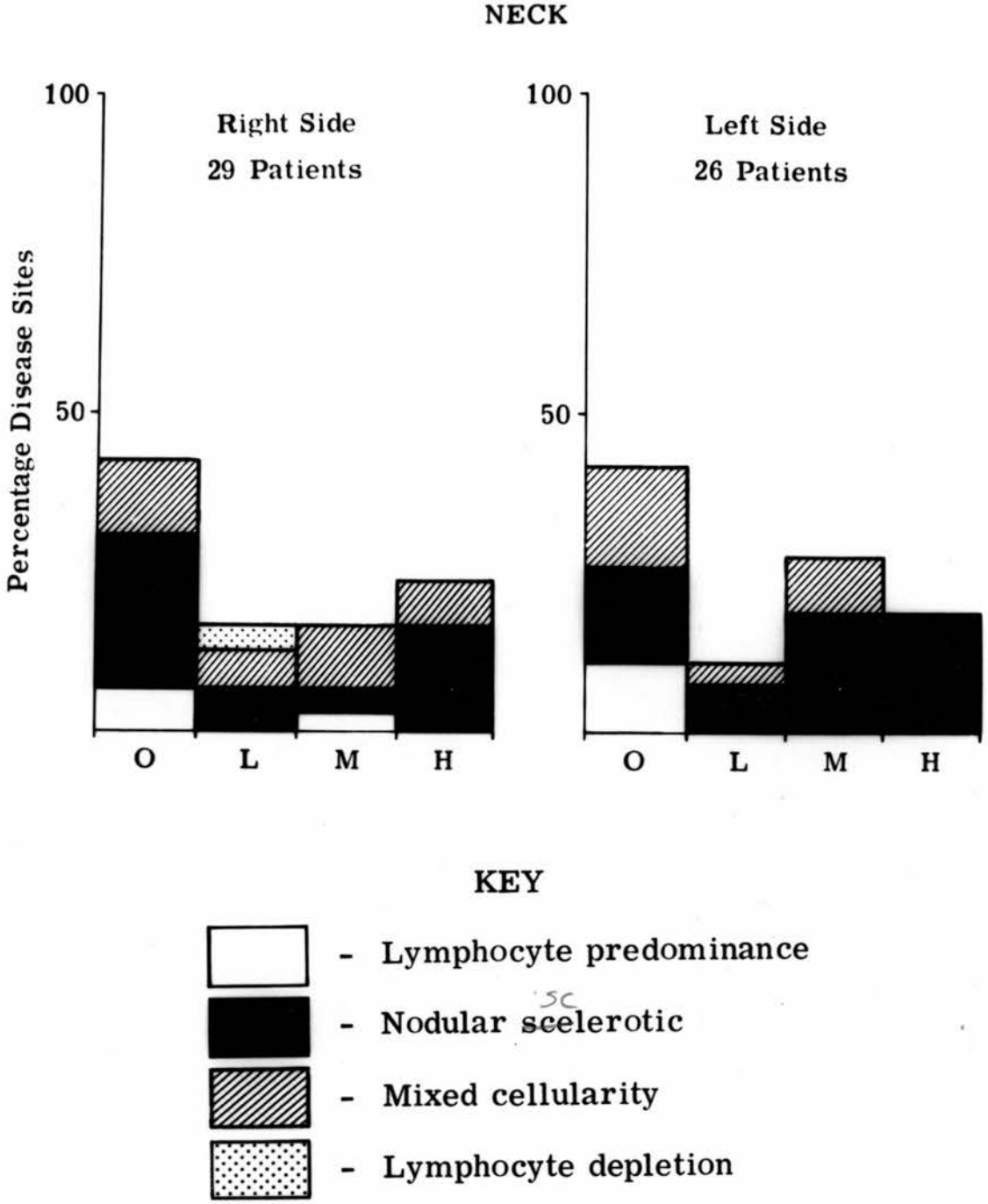
HISTOLOGY	% DISEASE AREAS SHOWING NO UPTAKE	% DISEASE AREAS SHOWING LIGHT UPTAKE	% DISEASE AREAS SHOWING MODERATE UPTAKE	% DISEASE AREAS SHOWING HEAVY UPTAKE	TOTAL % DISEASE AREAS SHOWING ANY UPTAKE
PHOCYTE DOMINANCE					
atients	81%	13%	6%	-	19%
disease areas	13	2	1		3
JLAR SCLEROTIC					
patients	35%	13%	25%	27%	65%
disease areas	51	19	37	40	96
ED CELLULARITY					
patients	40%	19%	21%	20%	60%
disease areas	28	13	15	14	42
PHOCYTE LETION					
atients	12%	25%	51%	12%	88%
isease areas	1	2	4	1	7

LE 1. The intensity of Gallium 67 uptake or complete absence of uptake at each anatomical site of ease has been compared with the histological sub-classification of each disease site, and expressed a percentage.

ble 3.1

Figures 3.1-6 show the ^{67}Ga imaging characteristics at different anatomical sites. It can be seen that the site of a lesion is an important factor in its imaging properties. The mediastinum and lung are the disease areas most successfully scanned giving 88% and 76% positive scans respectively. Disease in both sides of the neck and the left supraclavicular region was detected by ^{67}Ga in approximately 60% of all cases. Demonstration of disease in the right supraclavicular region, the axillae and the abdomen was less successful, approximately 40% of the known disease sites being positive.

The patients were then divided into two groups, the first group being previously untreated patients who were scanned on presentation, and the second group being a treated group who were scanned on relapse. (This was done in order to ascertain whether prior treatment affected imaging properties). The results of scanning the neck and supraclavicular regions could not be examined in this way because of the small numbers of patients relapsing in this areas. The ^{67}Ga uptake at the remaining sites was then reviewed. Detection of disease in the mediastinum showed no significant change. The abdominal nodes and the axillae showed a 30% increase in lesions imaged in treated patients while in the lung there was a 30% decrease in lesions imaged. Possible reasons for this are discussed later. When the disease areas are sub-divided into their various histologies, it can be seen that the "lymphocyte predominant" histology images poorly whichever area is scanned, and the "lymphocyte depleted"histology tends to image very well in any area.



g 3. 1-6

Levels of ^{67}Ga uptake related to known site of sease. Anatomical sites tabulated are neck (right & left), praclavicular regions (right & left), axillae, mediastinum ncluding hilar regions), lung, para-aortic and pelvic. The st five regions have each been sub-divided into previous eated and untreated groups. The levels of ^{67}Ga uptake used e described in detail in the methods section (p. 53). Briefly ey are: 0 = no uptake; L = Low (uptake less than liver); = Moderate (uptake equivalent to liver uptake); H = High ptake greater than liver uptake).

SUPRACLAVICULAR

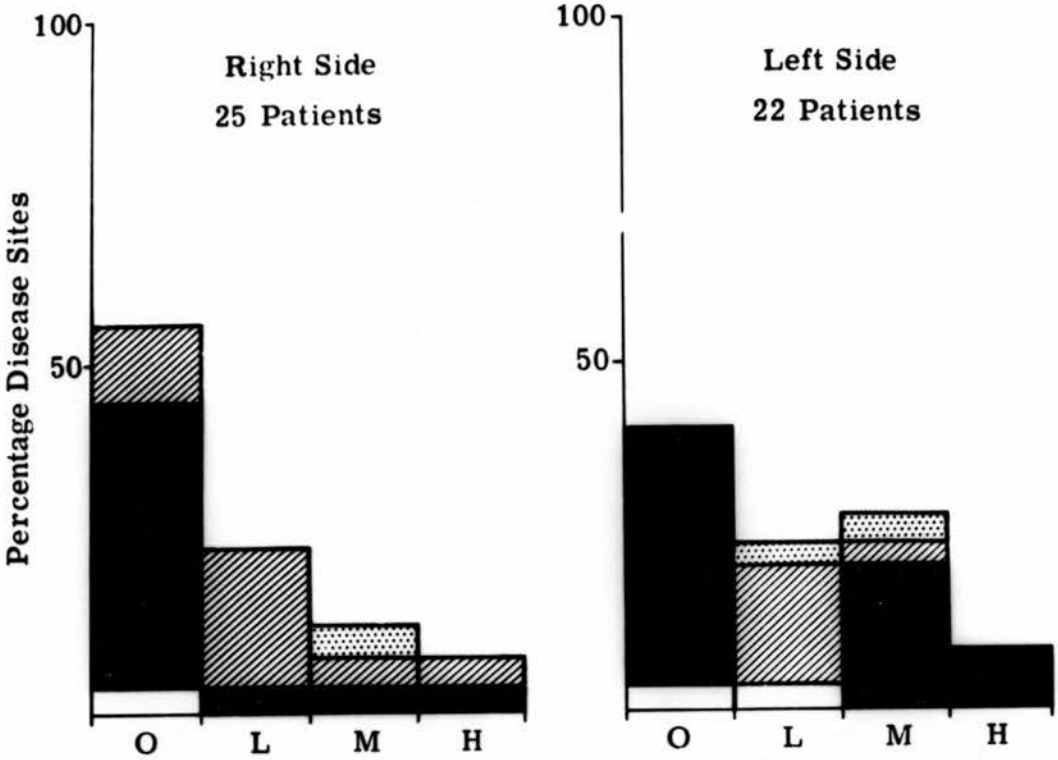
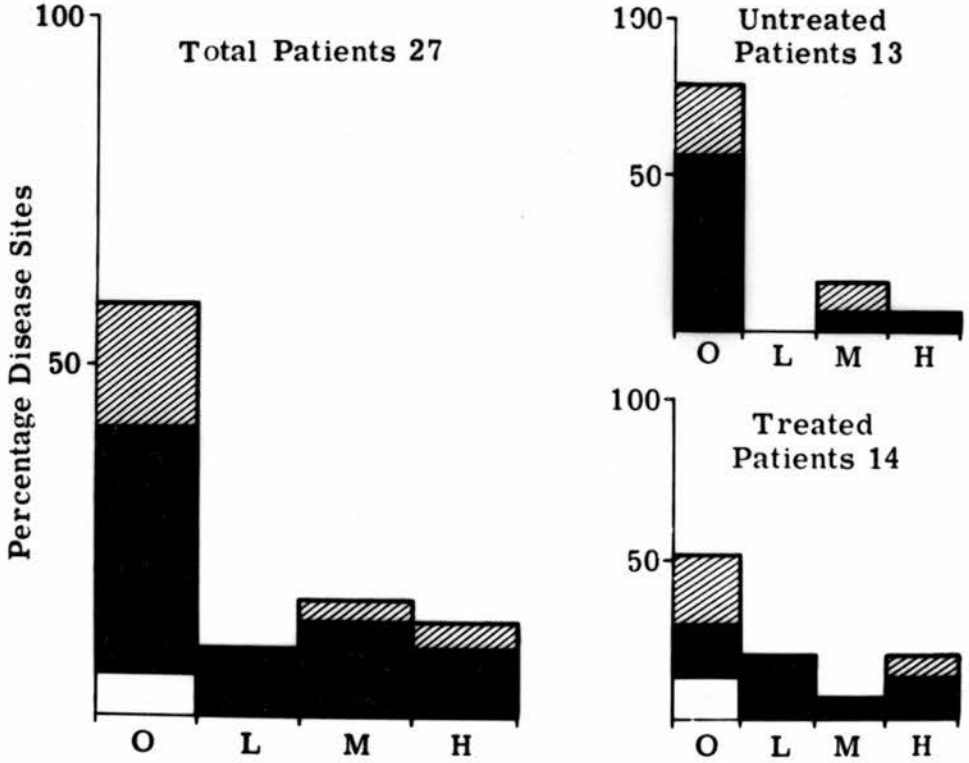


Fig 3.2

AXILLAE



g. 3 3

MEDIASTINUM AND HILUM

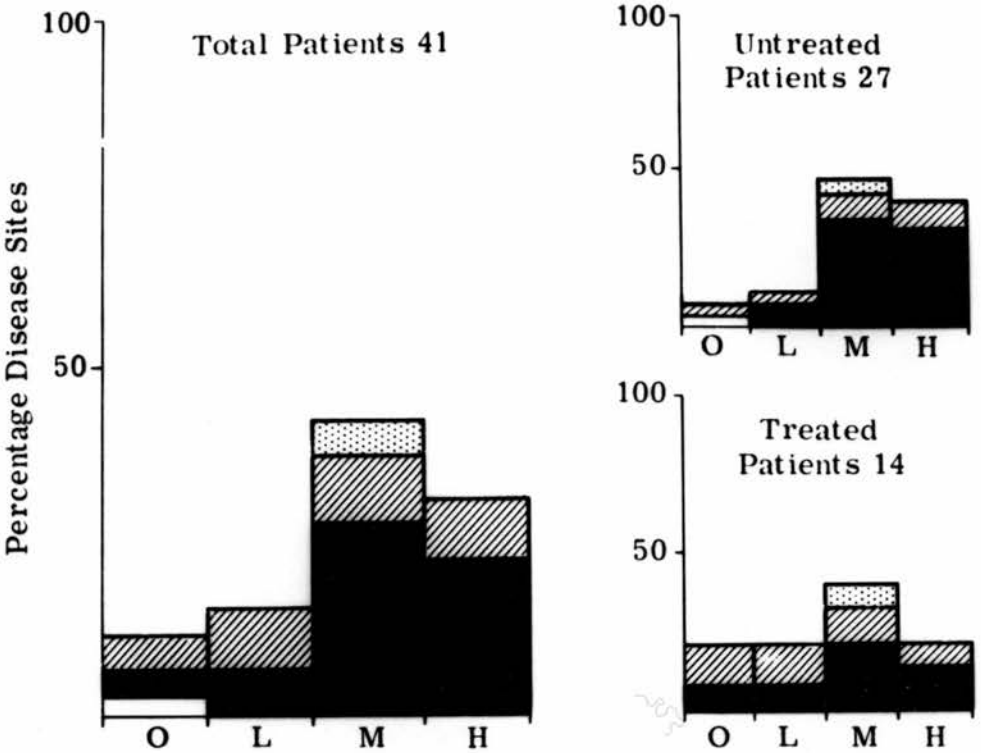


Fig. 3.4

LUNG

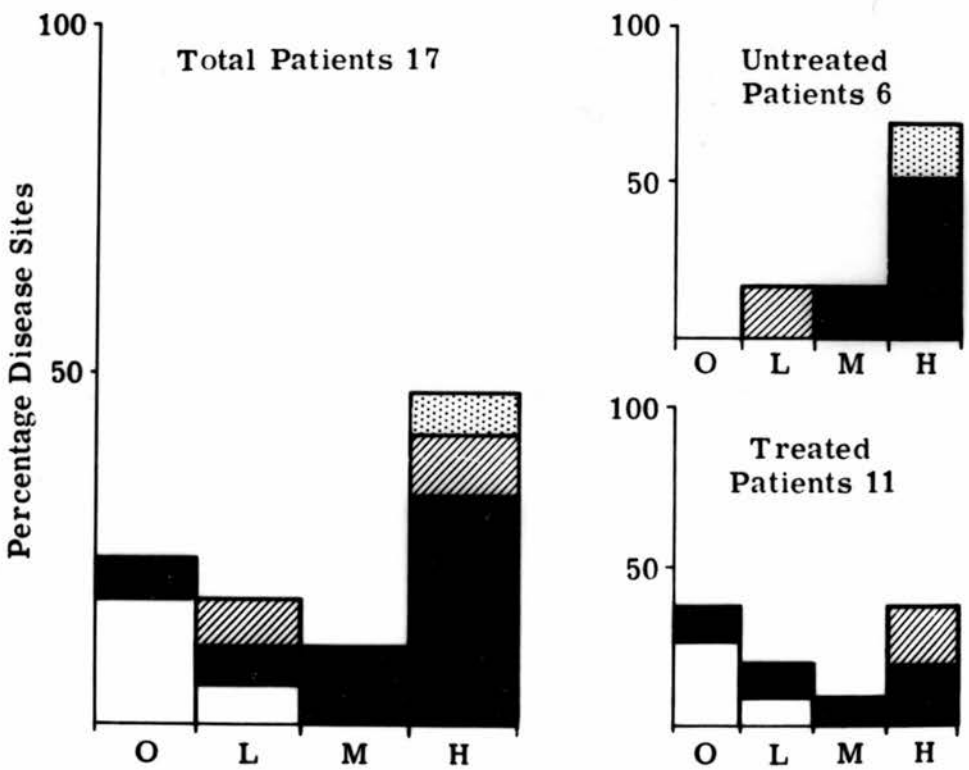


Fig. 3.5

PARA-AORTIC AND PELVIC

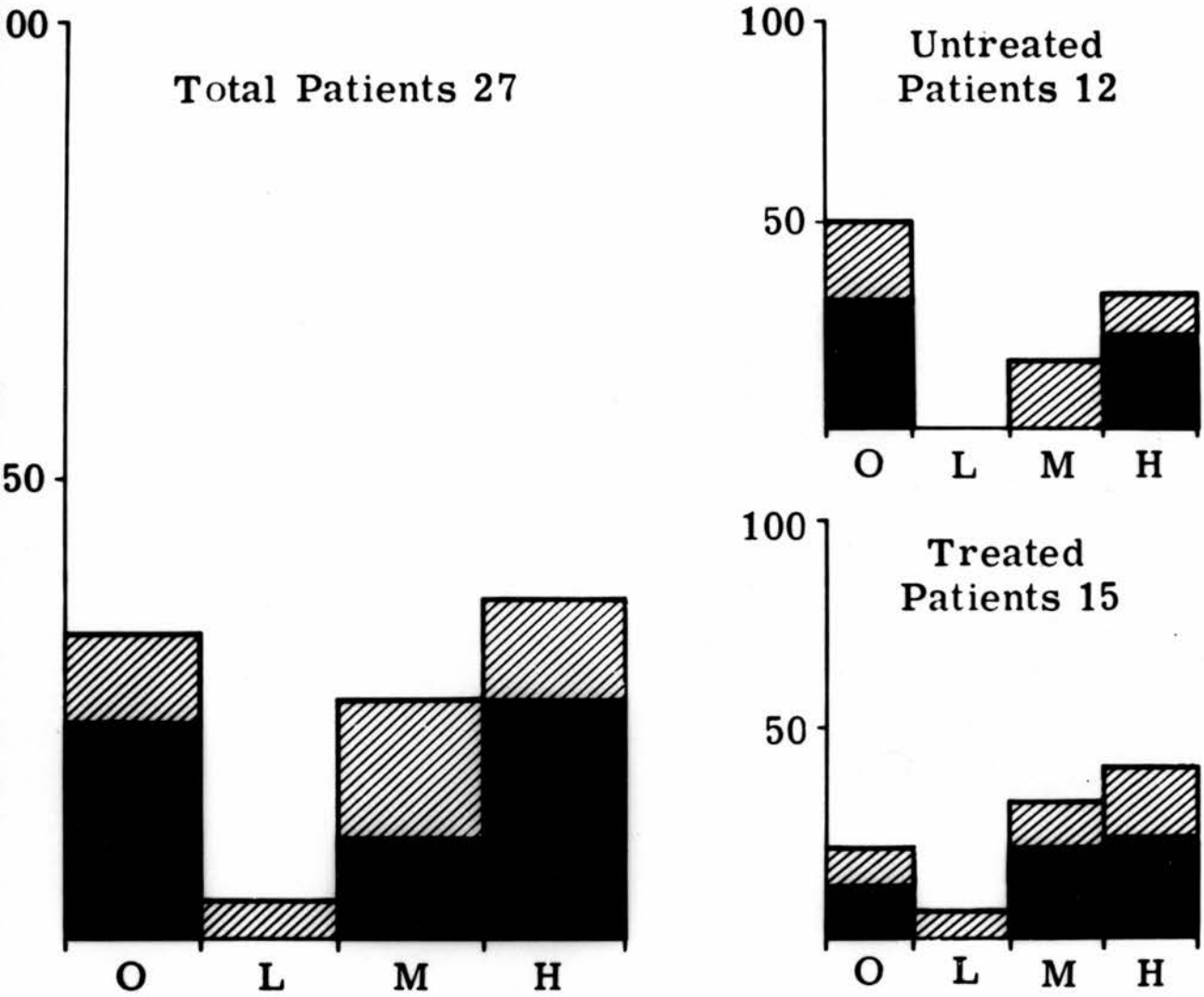


Fig. 3.6

Several other disease areas were also reviewed including the gut, bone, inguinal and infraclavicular nodes, but the number of patients so examined was too low to give any statistically valid information. The spleen was not studied because of difficulties in distinguishing between physiological and pathological uptake of ^{67}Ga.

The size of a mass of Hodgkin's disease might be thought to be important in determining whether or not that mass will image. Obviously, there is a lower limit beyond which disease is undetectable due to limitations of the technique. On investigating this possibility, we found great difficulties in estimating the size of involved disease areas from an analysis of the clinical notes because of the variations in methods of reporting or absence of relevant records. After attempting to relate the level of ^{67}Ga uptake to size of tumour mass using several different methods, no firm conclusions were reached. This aspect will be discussed later. Figure 3.7 is a graph plotting the stage of the disease against the level of ^{67}Ga uptake in each patient. Although there is no clear-cut correlation beteen stage of disease and level of uptake, there is a tendency for patients with Stage 1 disease to fail to image (7/13). It was found that 81% of all high uptakes were found in patients with widespread disease of Stage 11E or greater as compared to 48% with moderate uptake, 60% with minimal uptake and 38% with no uptake.

Results relating ^{67}Ga uptake levels to the length of remission and to patient mortality, are given in the remaining Figures and Tables.

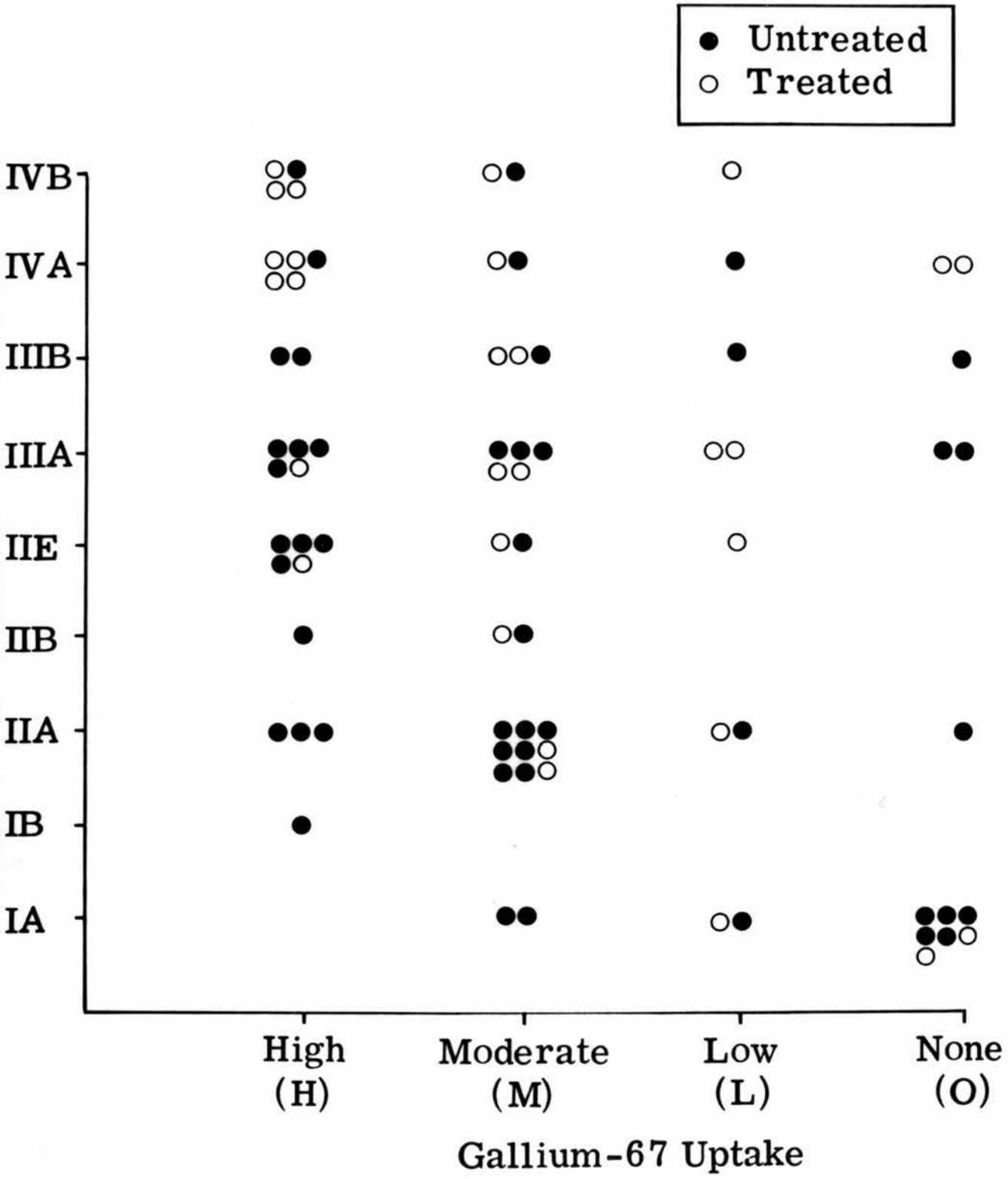

Untreated
Treated
IVB
IVA
IIIB
IIIA
IIE
IIB
IIA
IB
IA
High
(H)
Moderate
(M)
Low
(L)
None
(O)
Gallium-67 Uptake

Fig.3.7

Figure 3.8 shows the cumulative percentage remission rate of previously untreated patients with Hodgkin's disease for each level of ^{67}Ga uptake. Followup of the patients was carried out for up to two years after the initial remission was attained. There is a difference between the patients whose lesions showed either no ^{67}Ga uptake or low ^{67}Ga uptake, and those showing moderate or high ^{67}Ga uptake. None of the patients in the former group have relapsed. There is also a difference in the group showing moderate ^{67}Ga uptake compared to the high ^{67}Ga uptake group in that the moderate group appear to be doing better than the high uptake group at 6 months although by one year this difference is less significant.

A study of the remission lengths of those patients who were scanned on relapse is less informative from a clinical point of view because some of the patients in this group have relapsed more than once. The data has been presented in the form of a flow-chart (Figure 3.9) and again suggests that patients with a high level of ^{67}Ga uptake had shorter remissions than those with a low level of ^{67}Ga uptake or no uptake.

Table 3.2 shows all patients who were scanned during the terminal phases of their disease. Apart from one Stage 11E patient, all stages were either 111E or IV. The patients are grouped according to the level of ^{67}Ga uptake. The previous number of relapses before the scan and the lengths of remission following treatment after the scan are given. NR indicates no further remissions. It can be seen that most patients (80%) who did not remit exhibited a high ^{67}Ga uptake.

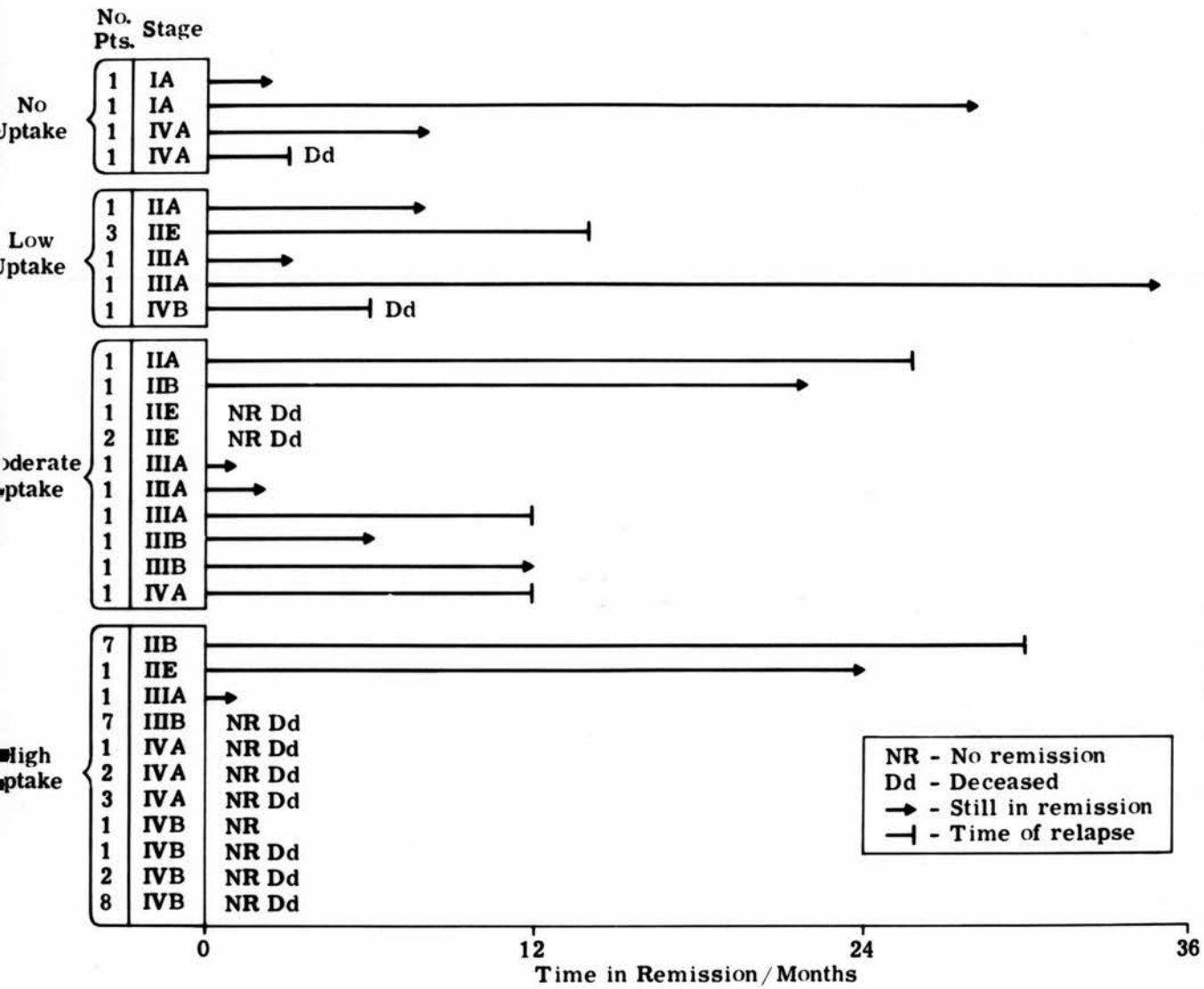

No. Pts.
Stage
No Uptake
1 IA
1 IA
1 IVA
1 IVA Dd
Low Uptake
1 IIA
3 IIE
1 IIIA
1 IIIA
1 IVB Dd
oderate ptake
1 IIA
1 IIB
1 IIE NR Dd
2 IIE NR Dd
1 IIIA
1 IIIA
1 IIIA
1 IIIB
1 IIIB
1 IVA
High ptake
7 IIB
1 IIE
1 IIIA
7 IIIB NR Dd
1 IVA NR Dd
2 IVA NR Dd
3 IVA NR Dd
1 IVB NR
1 IVB NR Dd
2 IVB NR Dd
8 IVB NR Dd
NR - No remission
Dd - Deceased
→ - Still in remission
⊣ - Time of relapse
0
12
24
36
Time in Remission/Months

Fig. 3.8

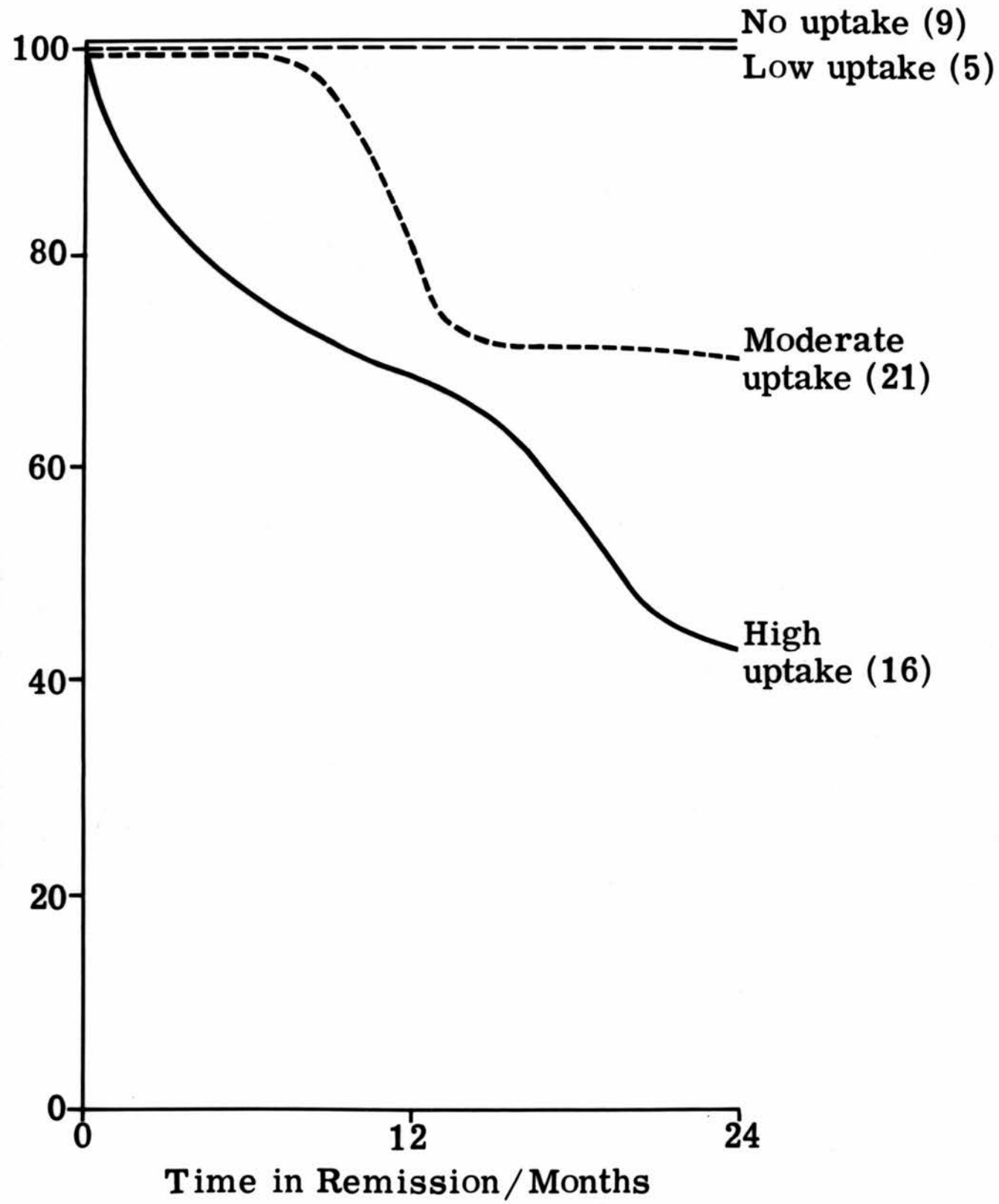

No uptake (9)
Low uptake (5)
Moderate uptake (21)
High uptake (16)
100
80
60
40
20
0
0
12
24
Time in Remission/Months

Fig. 3.9

DECEASED

Gallium Uptake	No. of Previous Relapses	Remission Length (months)	Survival (months)
O No Uptake	1	3	3
L Low Uptake	1	6	11
M Moderate Uptake	2	NR	2
	1	NR	21
H High Uptake	0	1	17
	0	NR	4
	1	NR	1 week
	1	NR	2
	2	NR	4
	2	NR	4
	3	NR	6
	7	NR	24
	8	NR	1

Table 3.2

These patients with high uptake had short survivals, most less than 4 months. The few patients with absent and minimal ^{67}Ga uptake achieved remission before death. The patient with no uptake died of cardiac failure while apparently still in remission.

DISCUSSION

Most studies of ^{67}Ga-citrate scanning in malignant disease have concentrated on scanning success rates in various types of tumour. There have been many reports assessing the clinical status of ^{67}Ga-citrate scanning in the management of Hodgkin's disease. The earliest reports (Kay and McCready 1972; Turner et al 1972) reported that 79% of regions subsequently shown to be involved with Hodgkin's disease could be imaged on scanning. The largest report has been from the "Co-operative Group to Study Localization of Radiopharmaceuticals" sponsored by the Oak Ridge Associated Universities (Johnson et al 1975). They studied 151 patients with Hodgkin's disease, finding that an overall 73% of proven disease sites were positive on scanning. The percentage of positive scans was highest in the neck and chest, and lowest in the abdominal and inguinal node regions. They also found that "scan detectability" of a lesion was not related to its size or to the presence of systemic symptoms.

It has been apparent for some time that there is a considerable variation in the visual level of ^{67}Ga uptake seen on scans in comparably sized lesions of the same

histology. Little attention has been paid to this variation in uptake, but the results of this retrospective study indicate that there may be some clinical significance in this phenomenon. Nash et al (1973) studied a series of colonic and rectal carcinomas and found some correlation of ^{67}Ga uptake with the degree of differentiation of the tumour, the poorly differentiated tumours taking up more ^{67}Ga than the well-differentiated tumours. It is possible that within each tumour group there is a range of uptake levels which might reflect the degree of malignancy. However, it is also clear that other factors are involved in the process of tumour imaging. Several workers have looked at ^{67}Ga uptake and histological sub-classification of Hodgkin's disease without coming to any firm conclusions, suffering from the same deficiencies as this study, namely, that of low numbers of patients. (Kay & McCready 1972; Johnson et all 1975).

Bearing in mind the low numbers of patients studied, it was rare to image a mass of lymph nodes of "lymphocyte predominant" histology. Lesions of "nodular sclerosis" or "mixed cellularity" gave an average of 60% positive scans. "Lymphocyte depleted" lesions were very successfully scanned, but again, the number of patients in this group is small.

The extremely good results from scanning of the lung and mediastinum confirm results from other centres. One cause of this may be related to low background activity in the chest, although this cannot be the whole answer, since background activity in the axilla is low, and scanning results in this area are poor.

Figure 3.7 shows no definite correlation between intensity of ^{67}Ga uptake and stage of disease. However, 7 out of 13 patients who had no ^{67}Ga uptake had Stage 1A disease. It might be felt that these patients were likely to have small neck lymph nodes, which would be difficult to image from the point of view of size. Individual assessment of the reported size of the glands did not confirm this, there being quite a range of sizes from large matted masses of lymph nodes to nodes 1 - 1.5 cms in diameter. It can also be seen that those patients with "B" symptoms tended to give more positive scans than those patients who were symptom-free.

It was a clinical impression that the very high levels of uptake of ^{67}Ga tended to occur in patients who were nearing death after having received a variety of treatments. This impression was strengthened by the finding that of 10 patients who were scanned shortly before death, 8 had scans showing a high level of ^{67}Ga uptake and 2 had scans showing a moderate level. No patients who died had low levels or no ^{67}Ga uptake.

In order to assess whether the level of ^{67}Ga uptake relative to the liver could be prognostically significant, it is essential to assess separately patients who have not received previous treatment and those who have. The results for the former group are shown in Figure 3.8. This figure, although being produced by standard actuarial methods (modified Wilcoxon test) must be interpreted with caution. There is a difference between the remission lengths of patients showing no uptake and low uptake and the groups showing moderate and high uptake, the low and absent uptake groups having longer remissions. The difference between the moderate uptake group

and the high uptake group is only slight except at the initial period of remission.

A flow-chart was constructed (Figure 3.9) of the remission lengths of relapsed patients, but less prognostic significance can be attached to it since this group comprises patients who have relapsed a variable number of times. However, this again suggests that heavy uptake of ^{67}Ga indicates a bad prognosis, since nearly all patients with heavy uptake had either short remissions or failed to remit.

This is the first report, investigating this aspect of uptake of tumour imaging radiopharmaceuticals in Hodgkin's disease. Cheguillaume et al (1974) found similar results investigating ^{57}Co-labelled bleomycin uptake in carcinoma of the bronchus. They measured tumour: background radioactivity ratios by means of a computer linked gamma-camera ("Intertechnique") and found a correlation between level of uptake and survival, patients with the "hottest" lesions surviving the shortest time.

These results suggest that patients who have lesions taking up ^{67}Ga avidly may be in a more aggressive phase of their illness or have a more aggressive disease than those whose lesions have visually low levels of ^{67}Ga uptake or fail to visualize.

3.2. SEMINOMA OF THE TESTIS

This section was published in the British Medical Journal, 1,1118-1121 (1976)

INTRODUCTION

The development of a satisfactory tumour localizing agent for the testicular tumours would be of considerable value from the point of view of choice of therapy, localization for external radiation and monitoring the response of the disease to treatment.

In seminomas, disease localization is of importance since the tumour is exceedingly sensitive to radiation, and sites which are usually vulnerable to radiation damage such as liver and lungs, can be irradiated to tumouricidal dose levels without prohibitive normal tissue damage. Thus, widespread disease is not necessarily incompatible with cure. In testicular teratomas there is the added problem of staging which is particularly relevant to a combined irradiation-chemotherapy approach for disease which has spread to the lungs and where it is important to exclude liver involvement.

The use of the tumour imaging agent ^{67}Gallium-citrate (^{67}Ga-citrate) in the whole-body scanning of patients with testicular tumours, has been assessed from 1972 onwards.

METHODS

2-3mCi of ^{67}Ga-citrate, obtained commercially from Philips-Duphar, was injected intravenously and 48 hours later the patients were scanned on a Selo Superscanner D.S.7. Bowel laxatives were routinely used prior to scanning, but if any suspicious area of activity

was seen in the abdomen, re-scanning 24 hours later was performed.

The tumours were classified in the Department of Pathology (Professor N. Gowing), Royal Marsden Hospital according to the classification put forward by the Testicular Tumour Panel and Registry (Collins and Pugh 1964).

RESULTS

23 patients with pure seminoma were scanned and received a total of 39 scans (Table 3.3). Of 15 scans performed at times when seminoma was subsequently confirmed to have been present by other methods or by response to treatment, 13 were positive in every disease site. There was one false negative scan occuring in a patient with small, but involved external iliac lymph nodes, which had been diagnosed on lymphography. One patient must be classified as a "partial" false negative. He had relapsed with bilateral hilar deposits with, in addition, one deposit in the right upper zone and another peripherally in the left lower zone. The Gallium scan was clearly positive in the right hilar and right upper zone deposits, but was negative for the left hilar and left lower zone deposits. The remaining 24 scans were performed on patients who were disease-free and were negative.

11 patients with a testicular teratoma were each scanned once and of nine who had disseminated disease with sizeable tumour deposits, only 2 patients showed light uptake in the disease areas. The other 2 patients scanned had Stage 1 disease and were negative (see Table 3.4).

Table 3.3

Seminoma of the testis

39 scans performed

	scan +ve	scan -ve
Tumour present	13/15	2/15
No tumour present	0/24	24/24

Table 3.4

Teratoma of the Testis

11 scans performed

	+ve scan	-ve scan
Tumour present	2/9	7/9
No tumour present	0/2	2/2

8 patients with combined seminoma/teratoma tumours were studied. These eight patients between them received 16 scans, one patient being scanned seven times (see table 3.5). Of 3 scans which were negative, no tumour was found with other investigations and this was confirmed by the patient continuing in remission clinically. Tumour masses were present at the time of the 13 other scans, and of these, five were performed on patients who had a tumour which was histologically thought to be, and clinically behaved like, a pure seminoma. These scans were positive in all disease areas. 2 patients subsequently developed teratomas, but unfortunately only one was rescanned. This one patient had a mediastinal tumour, which previously had imaged very well indeed with ^{67}Ga-citrate, but now could no longer be seen on scanning. Five patients with known combined tumours failed to respond to conventional treatment for seminoma and developed large masses of teratoma which also failed to image with ^{67}Ga-citrate.

We scanned one patient with a mixed teratoma-choriocarcinoma probably of mediastinal origin, and this tumour did image clearly with ^{67}Ga-citrate.

Two cases in the series should be discussed in more detail, in order to illustrate the value of Gallium scanning in seminoma of the testis.

Case 1. (See figure 3.11-13)

Patient Z.J. was found to have extensive intra-abdominal seminoma in 1969 for which he received radiotherapy. He relapsed in

Table 3.5

Combined tumours (Seminoma/teratoma).

16 scans performed.

Scan reflected the predominating tumour type.

Seminoma predominant	scan +ve	scan -ve
Tumour present	5/5	0/5
No disease present	0/3	3/3

Teratoma predominant

Tumour present	0/8	8/8
No disease present	0/0	0/0

1972 with deposits of seminoma in the right neck, para-aortic region, and prostate gland.

Chemotherapy was restarted and the patient went into complete remission until October of 1974 when he suffered from severe abdominal pain. From November of the previous year, ^{67}Ga-citrate scans had been performed at regular intervals as a follow-up procedure. An abnormal area of ^{67}Ga uptake was noted five months prior to the onset of symptoms, but at that time, because of lack of corroborative evidence, no action was taken (Figure 3. 11-12). A scan performed at the onset of his symptoms showed that the mass had enlarged in size (Figure 3.13). At operation an 8 cm. mass of tumour below the liver was found. No other investigation prior to laparotomy had indicated the presence of disease. The patient was put on chemotherapy and the mass was irradiated. He remained well until April of this year when a further area of abnormal uptake was noted in the left upper abdomen (Figure 3.14).

Case 2

This 37 year old patient had an orchidectomy for seminoma in the summer of 1972. There was no evidence of a combined tumour at that time. He was referred to the Royal Marsden Hospital in 1973 after relapsing in the mediastinum and was treated with radiotherapy. A Gallium scan was strongly positive in the mediastinum (Figure 3.15). He responded to treatment and the scan returned to normal. However, in 1974, he developed

a lower mediastinal mass which did not respond to treatment. This large mass failed to image with ^{67}Ga-citrate. He died from widespread deposits of teratoma. A tumour which was previously known to take up ^{67}Ga no longer did so, suggesting that the character of the tumour had changed.

Case 3

A 47 year old man presented with a tumour in the left testis in 1973. In September, 1974, he was referred to us complaining of backache and with evidence of extensive recurrent disease. A malignant teratoma was initially diagnosed, but, on review, the appearances were more suggestive of seminoma. There was radiological evidence of collapse of the 9th and 12th dorsal vertebrae and the first three lumbar vertebrae. There were also metastases in the right 10th and the left 5th and 6th ribs. The scan taken in September, 1974, (Figure 3.16A) showed increased uptake in the head of the left humerus, the dorsal and lumbar spine and in the ribs on both sides. There was also uptake in the ilium and right pelvic lymph nodes. After chemotherapy and radiotherapy the abnormal areas showing gallium uptake returned to normal. (Figure 3.16B). At the time of writing the patient remained well controlled on Cyclophosphamide.

DISCUSSION

Gallium scanning provides a convenient and safe means of whole body screening in the management of patients with seminoma of the testis.

Deposits of seminoma may arise in areas not easily accessible to other diagnostic techniques. Difference in uptake of ^{67}Ga in tumour sub-groups has not been emphasized. For example, the uptake characteristics of squamous cell carcinoma of the bronchus appear to be no different to "oat cell" carcinoma (Van der Schoot, Groen and de Jong 1972). The evidence presented in this report suggests that in the testicular tumours, ^{67}Ga may be more consistently taken up by seminoma than by teratoma. In the followup of patients who are suffering from what is presumed to be a pure seminoma, the change from a positive scan to a negative scan together with a change in the clinical course of the disease suggests that a teratoma may now dominate the clinical picture.

Pinsky et al (1973) have recorded the largest series of scans using ^{67}Ga-citrate in patients with testicular tumours. In this series only abdominal scans were done, and it was found that the method was useful in assessing the spread of tumour to the abdominal lymph glands. Embryonal carcinomas (anaplastic teratomas) tended to give positive scans, but there were no patients in the series who had disseminated seminomas and few who had more differentiated teratomas.

It is not known why seminomas, like lymphomas, have a high affinity for ^{67}Ga. Swarzendruber, Nelson and Hayes (1973) performed electron microscopic autoradiography on thymus and lymph node tissue of leukaemic mice and found that ^{67}Ga activity was predominantly in "lysosomal-like" granules within tissue macrophages. Inflammatory cells are known to have a high level of lysosomal activity. Seminoma is a tumour clinically characterized by a good response to treatment, and often histologically by

the presence of granulomata and lymphocytic infiltration, whereas teratoma of the testis is not generally characterized by either of these features. This may contribute to the particularly good imaging properties of seminoma.

The patient whose clinical details are summarized in Figure 3.11-13 demonstrates the value of this technique in the followup of patients with seminoma of the testis. The deposit of seminoma below the right lobe of the liver was noted six months before his clinical condition demanded exploratory laparotomy; the Gallium scan was the only test which detected the tumour recurrence. In Figure 3.14 a case history is given illustrating a difficult problem in the management of patients with seminoma of the testis. A patient who has been diagnosed as a pure seminoma and who then develops a recurrence in a previously treated area or who has responded poorly to standard treatment may have developed a teratoma and this diagnosis must be carefully considered in the above situations. In this particular case, a tumour which was previously known to take up ^{67}Ga no longer did so, and this suggested that the character of the tumour had changed. Figure 3.15 illustrates the value of Gallium scanning in the initial staging of a patient and as a monitor of the response to treatment.

In conclusion, this technique was found to be of great value in the diagnosis and followup of patients with seminoma of the testis. The apparent difference in imaging characteristics between seminoma and teratoma requires confirmation, and if proven, may have some value in the differential diagnosis of patients with combined tumours of the testis.

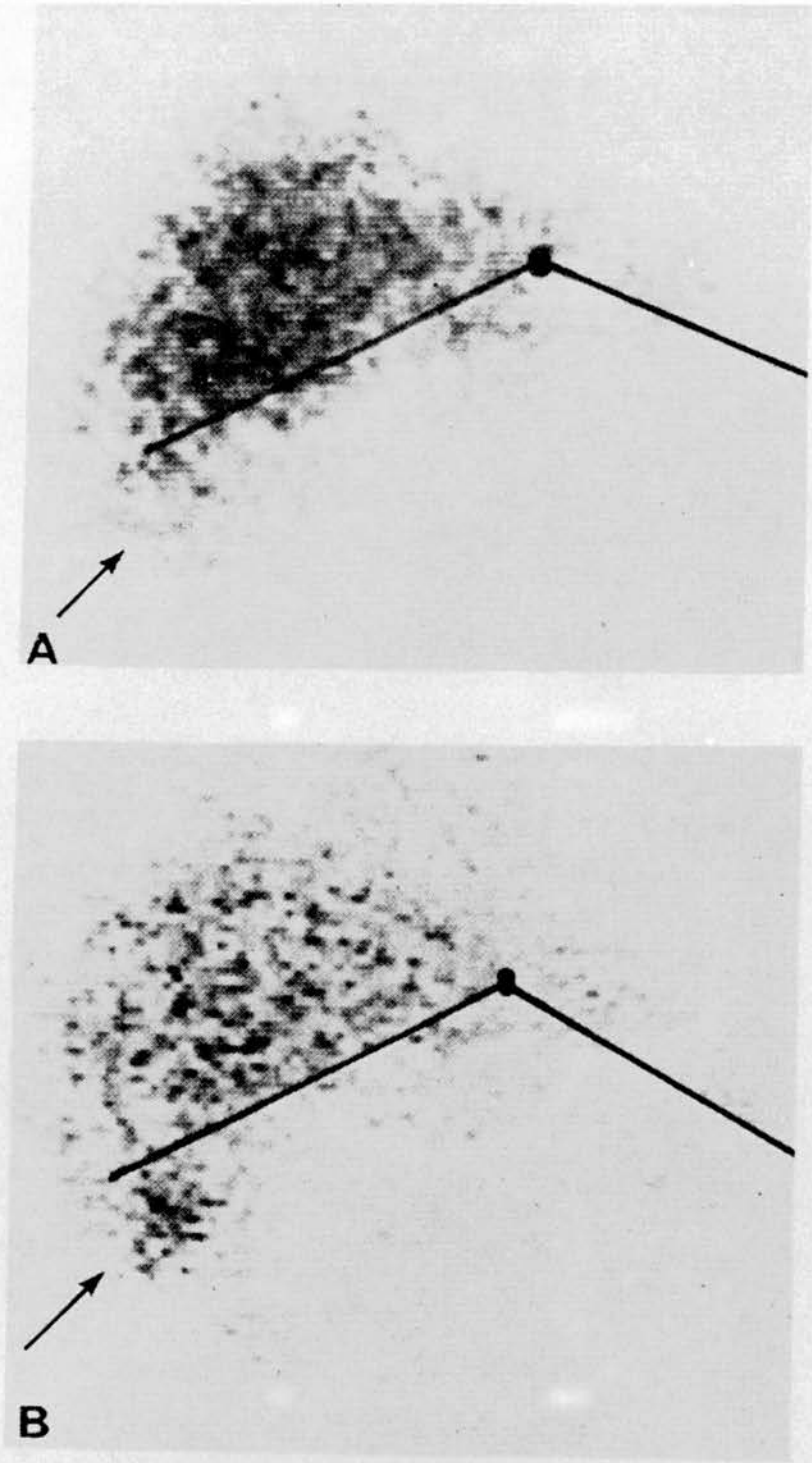
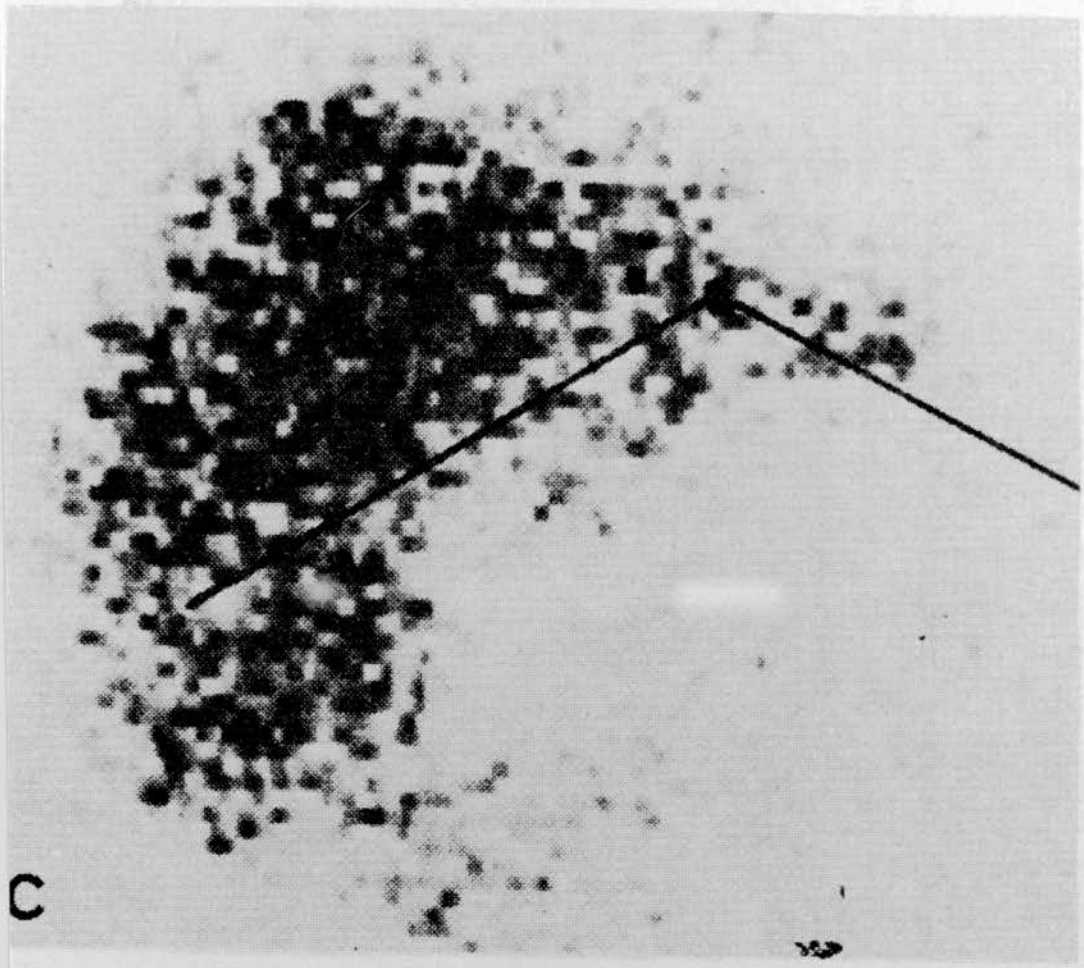
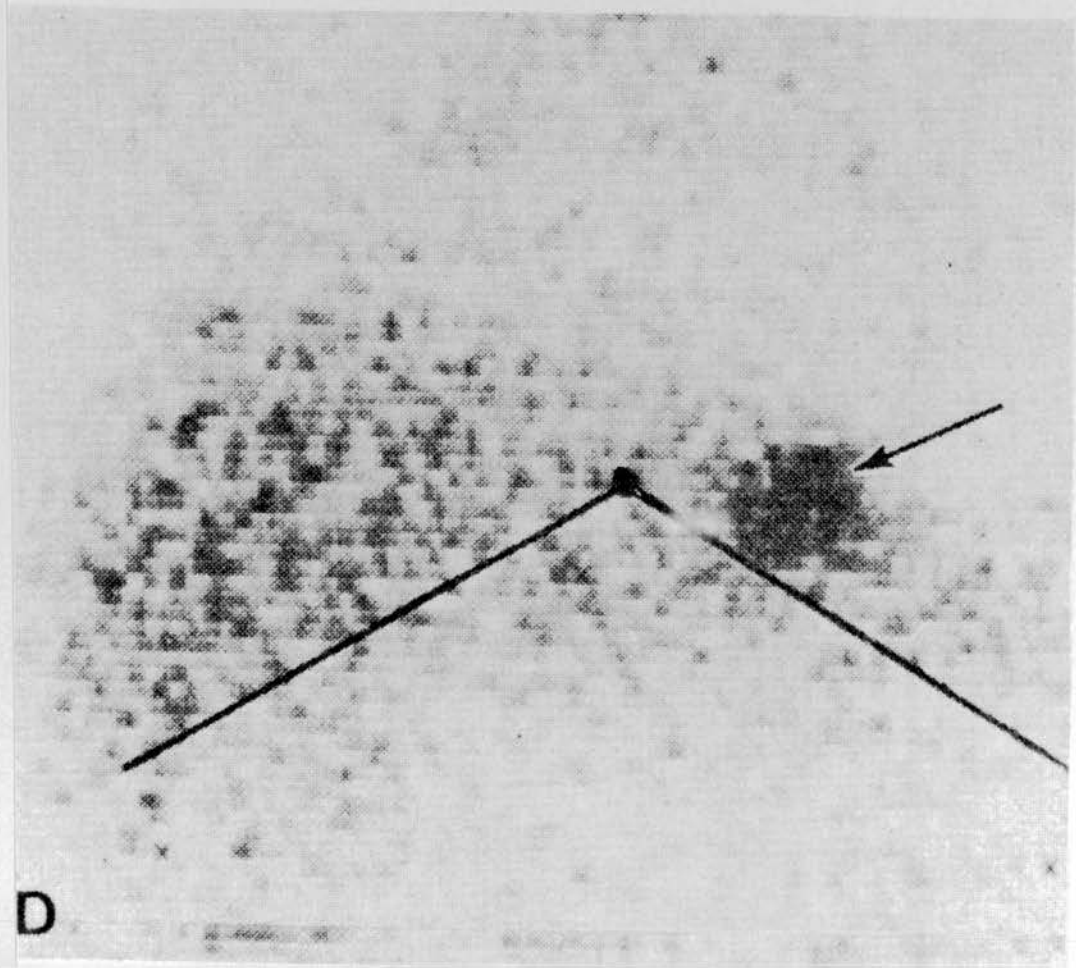


Fig. 3.11-12 –Case 1. Serial scans of upper abdomen. (A) December 1973. Normal liver image showing area where seminoma deposit later develops (arrowed). (B) May 1974. Abnormal area of ^{67}Ga uptake below right lobe of liver. (C) October 1974. Abnormal area has increased. Laparotomy revealed recurrent seminoma below right lobe of liver. (D) April 1975. Further recurrence in upper abdomen to left of midline (arrowed).

Fig 3.13-14





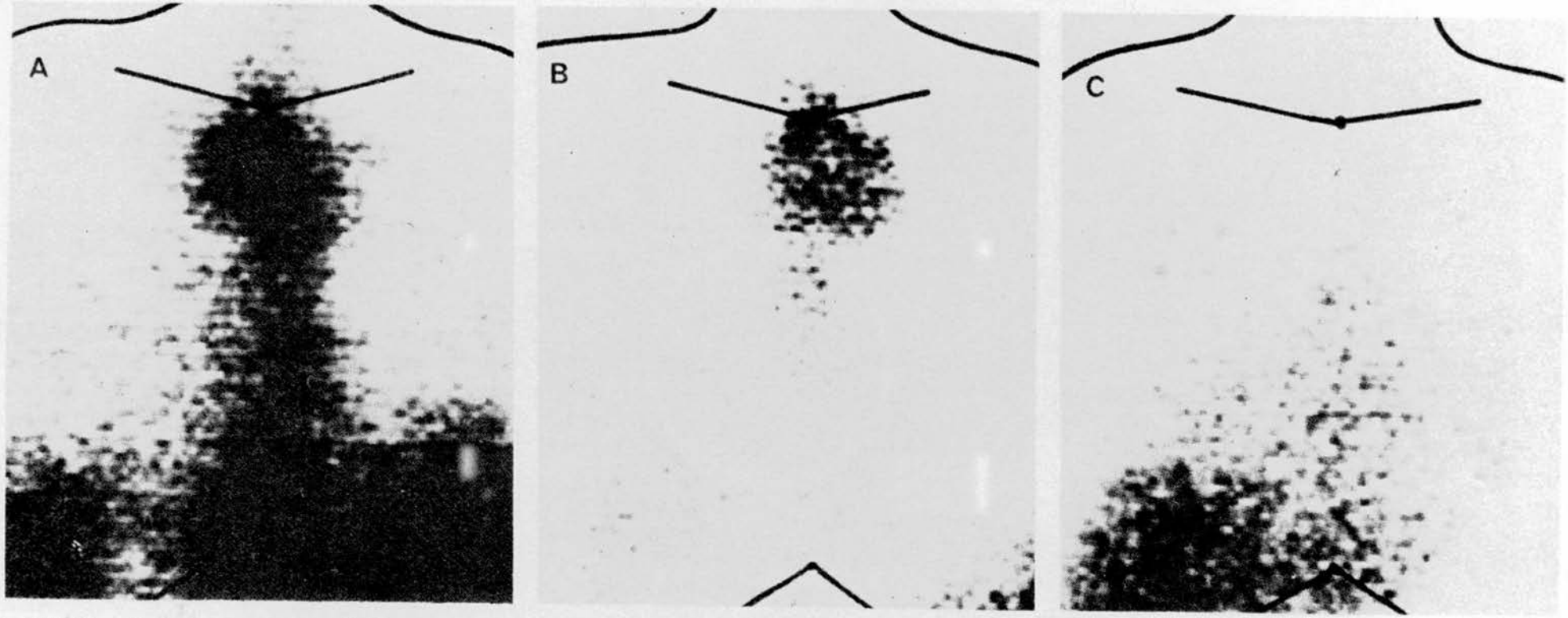


Fig 3.15–Case 2. Serial chest scans. (A) January 1972. Upper mediastinal mass of seminoma extends into right and left upper zone of chest. (B) February 1972 during radiotherapy. Mediastinal seminoma has shrunk and extends into left upper zone only. (C) March 1974. Large paravertebral and right lower zone deposit, which subsequently turned out to be teratoma. Only normal blood background can be seen and masses have not imaged with ^{67}Ga.

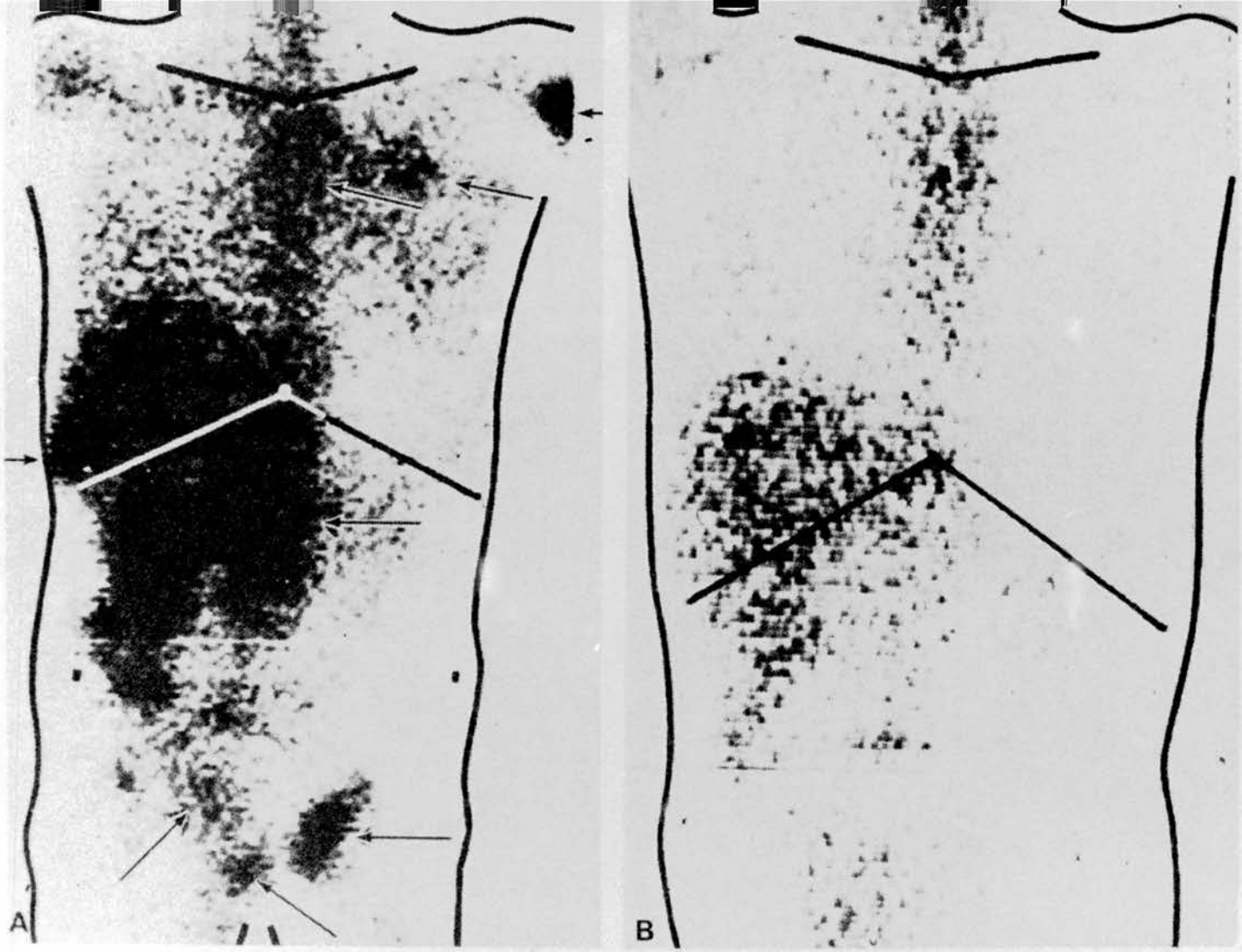


Fig 3.16—Case 3. (A) Before treatment for disseminated seminoma. Increased uptake at head of left humerus and in dorsal and lumbar spine, left ribs, right lower ribs, ileum, and right pelvic lymph nodes. There is also uptake in faeces in proximal colon, which disappeared on rescanning after a laxative. (B) After chemotherapy and radiotherapy these abnormal areas of ^{67}Ga uptake have returned to normal. Again, there is faecal uptake in the proximal colon.

4. MECHANISMS OF UPTAKE OF ^{67}Ga INTO TUMOURS

1. GENERAL INTRODUCTION

This section comprised part of an article published in the British Journal of Radiology, 48, 520-531 (July 1975)

MECHANISM OF UPTAKE

The mechanism of uptake of tumour-localizing agents is an intriguing and as yet, unsolved problem. Its elucidation will not only rationalize interpretation of clinical data, but may throw more light on the biology of tumours as a whole.

Most research on the mechanism of uptake has been done on ^{67}Ga-citrate. It has been shown that ^{67}Ga given as ^{67}Ga-citrate intravenously is bound to plasma protein (Gunasekera, King and Lavender, 1972), predominantly in the β-globulin fraction and, within this, transferrin appears to be an important carrier. In addition, a haptoglobin peak can be seen on immuno-electrophoretic autoradiography. However, 70 per cent of the scanning dose in patients appeared to be loosely associated with albumin and some globulins.

It is the mode of uptake of the radionuclide into the tumour which is proving difficult to explain on a rational basis. Using light and electron microscopic autoradiography, Swartzendruber, Nelson and Hayes (1973), examined the cellular and sub-cellular distribution of ^{67}Ga in a variety of tissues in both leukaemic and normal AKR* mice. They found that ^{67}Ga localization in the normal mice occurred mainly in the macrophages of lympho-reticular tissues, thymic epithelial reticular cells, Kupffer cells, hepatic cells

* A.K.R. = Strain in-bred at Rockefeller Institute, 1936. High leukaemic incidence.

and cells of the proximal convoluted tubules of the kidney. In the leukaemic mice heavy uptake occurred in the thymus and was related to increased numbers of macrophages. The leukaemic cells, although taking up ^{67}Ga, were not so heavily labelled as the macrophages. The electron microscopic autoradiographic pictures showed that 60% of the intracellular ^{67}Ga was associated with "lysosomal-like" granules in the cell cytoplasm. Others who have used biochemical cell fractionation techniques have not supported this E-M autoradiographic data. Becker et al (1972), performing ultracentrifugation of homogenized normal mouse liver and Erhlich ascites tumour cells, found that the ^{67}Ga distribution within the various cell fractions was greatest in the cytoplasmic supernatant fraction. In normal liver cells, 33% of the ^{67}Ga was in the cell cytoplasmic fraction, and in the ascites tumour cells, the proportion was 61 per cent. Very little radioactivity was found in the lysosomal fraction. These results have been supported by Orii (1972). However, the proposition that ^{67}Ga localization occurred on or within lysosome-like granules was supported by Haubold and Aulbert (1972) who found that using ultracentrifugation of rat liver homogenate, the 6,500 - 9,000 G sediment (the lysosomal fraction) contained 24 times the radioactivity of the rest of the cell fractions. It is possible that variations in the time interval between ^{67}Ga-citrate injection and the fractionation work would account for these differences.

The thesis that ^{67}Ga is taken up by the lysosomal-rich phagocytic cells of the reticulo-endothelial system is an attractive one. Recruitment of macrophages is known

to occur in certain growing animal tumours (Metcalf, Ishidate and Brumby, 1967), and the idea of ^{67}Ga accumulation within a tumour occurring as a result of the presence of macrophages within the tumour substance would be of immense significance both clinically and from the research point of view. Research on this aspect will be described in a later section.

Others have suggested that ^{67}Ga uptake may be related to the rate of cellular proliferation. Using an ascitic plasmacytoma (J.B.-1) and normal bone marrow cells in Balb/C strain of mice, Bichel and Hansen (1972) showed that in the rapid growth phase of the ascitic tumour, there was a higher intracellular ^{67}Ga uptake than in the tumour's plateau growth phase. Furthermore, normal mouse marrow made hyperplastic by prior bleeding of the mice took up ^{67}Ga more than hypoplastic marrow cells. However, it must be said that although ^{67}Ga uptake may be related to the rate of cellular proliferation in the mouse plasmacytoma and normal mouse marrow, it cannot be assumed to be true for other types of normal or malignant tissues. Hammersley and Taylor (1974) have suggested that although there is some form of relationship between uptake of ^{67}Ga and the rate of DNA synthesis, the uptake of the radionuclide is not simply related to the rate of DNA

synthesis *per se*. They examined two mouse tumours in detail, the Harding-Passey (HP) melanoma and the ADJ/PC6 plasma cell tumour. Three H-P melanomas of different sizes were examined. In the smallest tumour, there was a negative correlation between ^{67}Ga uptake and the rate of DNA synthesis, but in the larger two tumours examined, there was a positive correlation. These tumours were treated with radiation or cyclophosphamide, thus considerably reducing the rate of DNA synthesis. There was, however, no decrease in the ^{67}Ga uptake. Indeed, in two ADJ/PC6 tumours, there was increased uptake.

The research into mechanisms of uptake described in this thesis has been approached from three angles. Firstly, the macrophage uptake theory has been investigated; secondly, the contribution of vascular permeability to tumour uptake of ^{67}Ga has been investigated; thirdly, research into the similiarities and differences between the behaviour of ^{67}Ga and ^{45}Ca is described. One of the great impediments to rationalization of cancer chemotherapeutic regimes in humans is the sparsity of suitable methods of measuring tumour response to treatment. We are limited to observing whether a tumour gets bigger or smaller, which often takes a long time to show, and to measuring certain tumour products in the blood, such as

ectopic hormones. Furthermore, it might be possible to improve the specificity of tumour localizing agents if the uptake mechanism were understood.

4.2 THE CONCENTRATION OF ^{67}Ga and ^{45}Ca IN THE LACTATING MAMMARY GLAND AND ITS RELEVANCE TO THE TUMOUR UPTAKE OF ^{67}Ga-CITRATE

This section was published in Cancer Research, 36, 452-457 (February, 1976)

INTRODUCTION

The detection of malignant tumours by whole-body scanning after the injection of a radiopharmaceutical which has the property of localizing within a tumour is an attractive diagnostic method. However, this technique is in its early stages of development and, in order to improve tumour imaging, higher tumour:background radioactivity ratios must be achieved. To this end, the study of the mechanisms of tumour uptake and retention of these agents and their systemic metabolism may lead to improvements in imaging.

It is becoming clear that the phenomenon of tumour uptake of radionuclides is not restricted to one or two substances, but is a far more general phenomenon, embracing a wide variety of radiopharmaceuticals. One of the commonest in clinical use is ^{67}Ga-citrate which concentrates in a variety of tumours. It is not a tumour-specific agent, and it will concentrate within abscesses and other infective processes, and also in certain granulomatous diseases such as sarcoidosis. Physiologically, it is taken up in significant amounts by the liver and spleen, small and large intestine, and bone marrow. However, in addition, striking physiological accumulation occurs within the breasts of pregnant or postpartum women. It has also been

observed in a young woman with Hodgkin's disease who was neither pregnant nor postpartum. On this occasion, the breast uptake was associated with lactation due to hyperprolactinemia possibly caused by chlorpromazine or by cytotoxic chemotherapy. (See Figure 5.1)

The mechanism by which ^{67}Ga accumulates either in tumours or in the lactating mammary gland is not fully understood. Larson and Schall (8) measured the ^{67}Ga concentration in milk expressed from the breasts of one of their patients and found that ^{67}Ga was being secreted in significant amounts in the milk. Fogh (5) also measured the concentration of ^{67}Ga in breast milk and found that is was approximately the same as the blood ^{67}Ga activity, concluding that imaging of the breasts was unlikely to be due to ^{67}Ga accumulation within milk.

To obtain more information on the mode of uptake of ^{67}Ga in breast tissue and its possible relationship to the mode of tumour uptake, ^{67}Ga-citrate was compared to ^{45}Ca, a radionuclide that has been extensively studied in relation to milk biochemistry.

METHODS

In the clinical study (Fig. 5.1), the patient was given 2.5 mCi of ^{67}Ga-citrate (Philips-Duphar NV, Netherlands) I.V. and was scanned 48 hours later with a Selo Superscanner DS7.

Figure 5.1

This photoscan of the chest and upper abdomen of a young woman with dgkin's disease shows increased ^{67}Ga uptake at the left hilum, where there s a large involved node, and in the breasts. Normal uptake is also seen in e liver and bones. This patient had received quadruple chemotherapy with lorpromazine sedation 1 week prior to the scan. A scan taken before treatnt had started showed uptake in the left hilar region only. A further scan week later showed that ^{67}Ga uptake in the left hilar region had nearly turned to normal but that uptake in the breasts had further increased asma prolactin levels were found to be raised during chemotherapy and return- to normal after chemotherapy.

ANIMAL STUDIES

The dogs, 2 lactating bitches and a dog bearing a transmissible venereal tumour, received by i.v. injection 1mCi of carrier-free ^{67}Ga citrate plus 500 mCi of $^{45}CaCl_2$ (The Radiochemical Centre, Amersham, Endland; specific activity, 10 to 40 mCi/mg). Blood and milk samples were collected at intervals over a period of 100 hours. Biopsy samples of mammary tissue were removed at 5 and 48 hours after the injection of the radionuclides. Biopsy samples of tumour were removed at approximately 24, 48 and 72 hours after injection of the radionuclides. All biopsy material was placed on ice immediately after removal and analyzed within 2 - 4 hours. The animals were anaesthetized with halothane, following induction with thiopentone and premedication with acetylpromazine.

The samples of mammary gland and tumour tissue were cut into multiple small pieces which were weighed and assayed for ^{67}Ga and ^{45}Ca. Other samples of gland and tumour were weighed and the glandular tissue was rinsed in ice-cold 0.25m sucrose to remove any adhering milk. They were then homogenized in 0.25m sucrose and subjected to subcellular fractionation by centrifugation at 400 x g for 10 mins. to sediment nuclei + cell debris; at 6,000 x g for 15 mins. to sediment a heavy mitochondrial + lysosomal fraction; at 30,000 x g for 25 mins. to sediment the light mitochondrial + lysosomal fraction, and at 105,000 x g for 60 mins. to sediment the microsomal fraction and yield the soluble cytosol fraction. The pellets were taken up in distilled water and assayed for protein and

the marker enzymes, aryl sulfatase (lysosomes) and succinate dehydrogenase (mitochondria), using methods previously described (Dodgson, Spencer and Thomas, 1955; Pennington 1961.)

The radioactivity due to ^{67}Ga in the tissue, tissue fractions, plasma, and milk samples was assayed by γ - ray spectrometry in an automatic γ - ray spectrometer. For the assay of ^{45}Ca, the samples were placed in glass liquid scintillation counting vials, and 1ml 72% perchloric acid and 1 ml 30% (w/v) hydrogen peroxide were added: the mixture was allowed to stand overnight at room temperature. The samples were then heated at 70° for 2 to 4 hours until a clear, normally colourless solution was obtained (Seidel and Volf, 1972), and the vials were capped and stored for 30 days to allow the ^{67}Ga to decay. After storage 3 ml. of water and 10 ml. of an emulsion scintillant comprising 2 parts Tergitol TP9 (Union Carbide Co., Ltd., London, England) and 1 part 6% butyl-PBD in toluene were added, and the vial was shaken well to mix the contents thoroughly. The resulting gel was assayed for ^{45}Ca in an automatic liquid scintillation spectrometer. The correction for quenching was made by means of the external standard channels ratio method.

To determine the distribution of ^{45}Ca and ^{67}Ga between the protein and non-protein components of the milk, samples of milk were layered onto the top of a column of Sephadex G-75 (2.5 cm. in diameter and 10 cm. high) and eluted with 0.1 m Tris-HCL in 0.14 mKCl buffer at pH 7.4. Five-ml fractions were collected on an automatic fraction

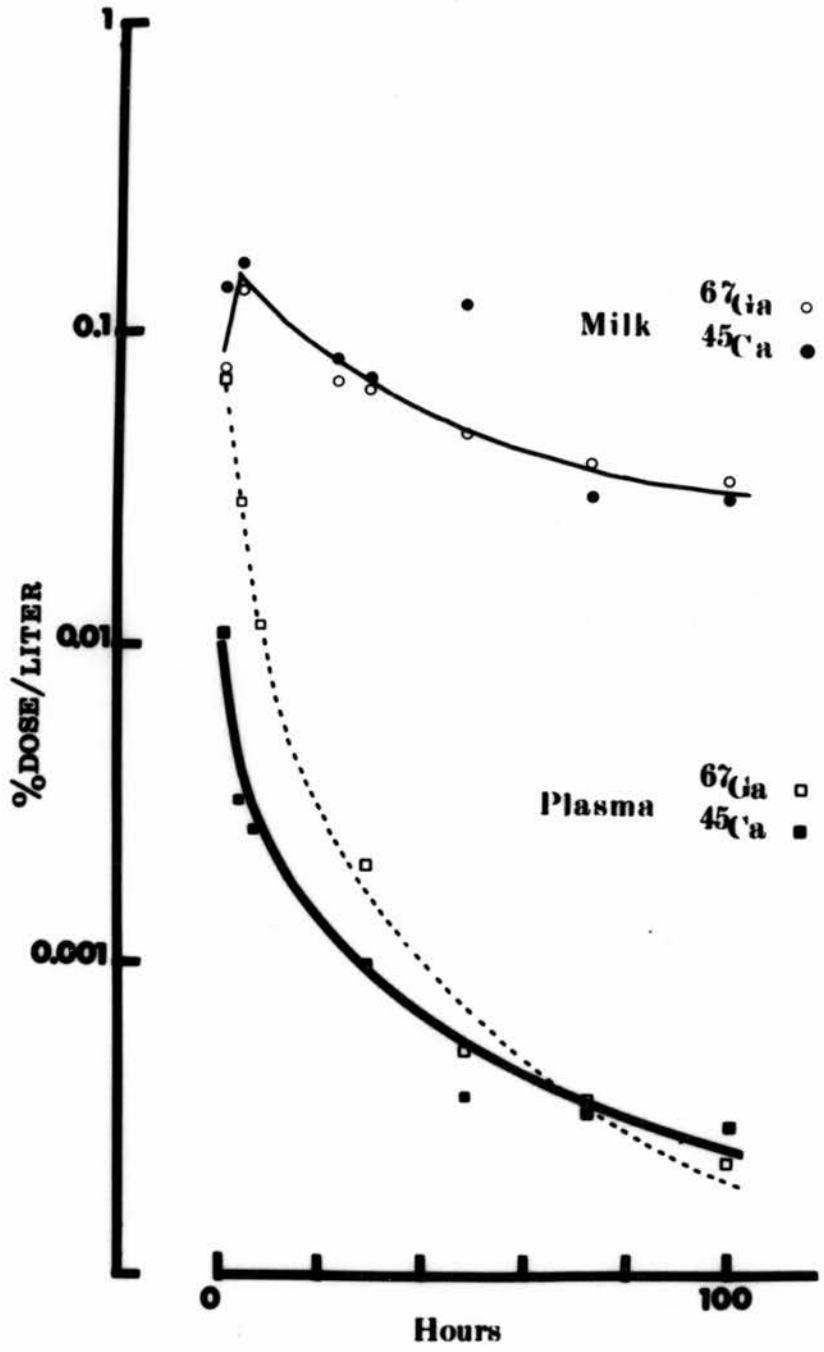
collector, and these fractions were assayed for ^{45}Ca, ^{67}Ga and protein.

RESULTS

The relative concentrations of ^{67}Ga and ^{45}Ca in milk and plasma over a period of 100 hr. following the injection of ^{67}Ga-citrate and ^{45}Ca-chloride into a lactating dog are shown in Figure 5.2. As can be seen from the data, there is a rapid fall in the plasma level of both ^{67}Ga and ^{45}Ca, while the concentrations of the 2 nuclides in the milk rises to a peak within one hour and then falls relatively slowly. Thus, there appears to be an early and substained concentration of both ^{67}Ga and ^{45}Ca in the milk, compared with the plasma. The concentrations of both nuclides are very similiar in all the milk samples examined, but the initial rate of clearance of ^{45}Ca from the plasma is more rapid than that of ^{67}Ga.

Milk samples collected between 4 and 7 hours after injection were fractionated on Sephadex G-75, and similiar elution profiles were observed for each of the samples examined. A typical elution profile is presented in Figure 5.3. This shows that almost all the ^{45}Ca and ^{67}Ga are eluted with milk proteins at the void volume of the column. Small amounts of both nuclides, about 5% of the total activity, were eluted in Fraction 5; this fraction corresponds to the elution peak of $^{45}CaCl_2$ and ^{67}Ga-citrate under the experimental conditions used.

The subcellular distribution of ^{67}Ga and ^{45}Ca was studied in samples of lactating mammary gland removed at 5 and 48 hr. after



5.2. The concentration of ^{67}Ga and ^{45}Ca in dog plasma and milk following simultaneous I.V. injections of ^{67}Ga citrate and $^{45}CaCl_2$.

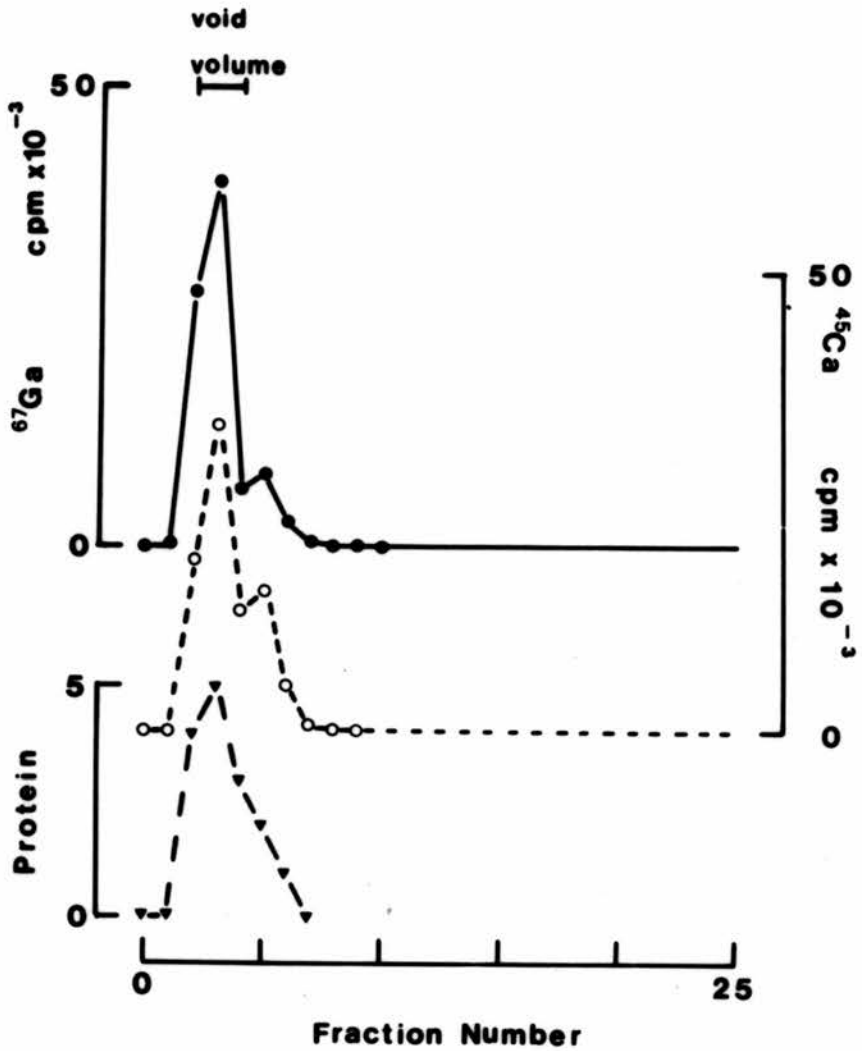


FIGURE **5.3** .

Elution profiles of ^{67}Ga (●—●), and protein (▼—▼) following fractionation
og milk in Sephadex G-75. Protein concentration is expressed in arbitrary
s.

injection of the nuclides. The results of these studies are illustrated in Figure 5.4 in which the relative specific activity of the nuclide of the marker enzyme in each fraction is plotted against the percentage present in that fraction. Relative specific activity is defined as the percentage of the total ^{67}Ga, ^{45}Ca, or enzyme in the fraction; using this method of presentation, the area of each rectangle on the diagram is proportional to the percentage of the total activity present in each fraction. From the data shown in Figure 5.5, it can be seen that, at 5 hr. after injection, the relative specific activity of ^{45}Ca is more or less constant and equal to unity in each fraction, with the exception of the heavy mitochondrial-lysosomal fraction. However, with ^{67}Ga, the relative specific activities in the mitochondrial-lysosomal fractions and in the cytosol are greater than those in the nuclear or microsomal fractions. At 48 hr. after injection, the ^{67}Ga and ^{45}Ca activity was found predominantly in the lysosomal fraction. At the early time interval 56% of the ^{67}Ga and 53% of the ^{45}Ca was present in the cytosol and, at 48 hr., about 40% of the total mammary gland content of each nuclide was still present in this fraction. In other studies a single sample of nonlactating human breast tissue was subjected to subcellular fractionation and 61% of the total ^{67}Ga was found in the cytosol at 48 hr. after injection.

The subcellular distribution of ^{67}Ga and ^{45}Ca was also studied in the transmissible venereal tumour at 80 hr. after the injection of the nuclides, and the results are shown in Figure 5.6. In this tumour, considerable amounts of ^{45}Ca (61%) and ^{67}Ga (40%) were found in the

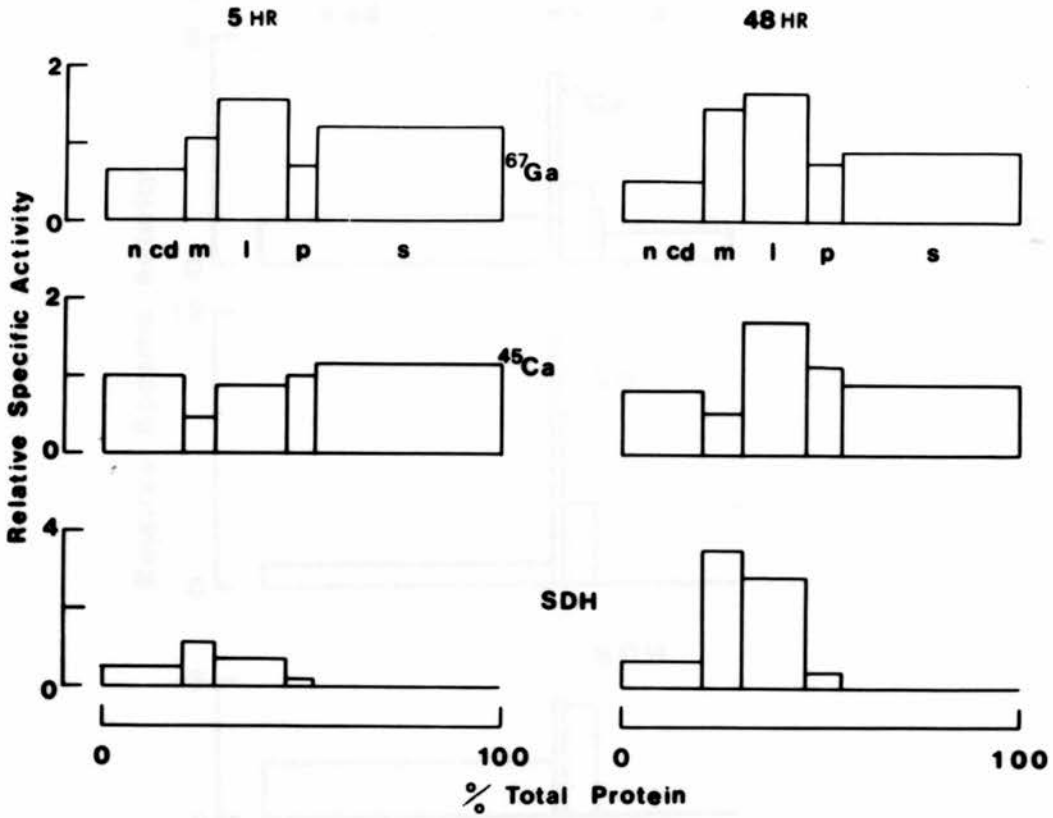


FIGURE **5.4.**

The subcellular distribution of ^{67}Ga, ^{45}Ca, and succinate dehydrogenase) (mitochondrial marker enzyme) in breast tissue taken from dogs at 5 48 hr. after i.v. injection of ^{67}Ga citrate and $^{45}CaCl_2$. Relative ific activity is defined as the percentage of the total protein in that tion. ncd, nuclei-cell debris faction; m, heavy mitochondrial lysosomal tion; p, microsomal fraction; s, cytosol.

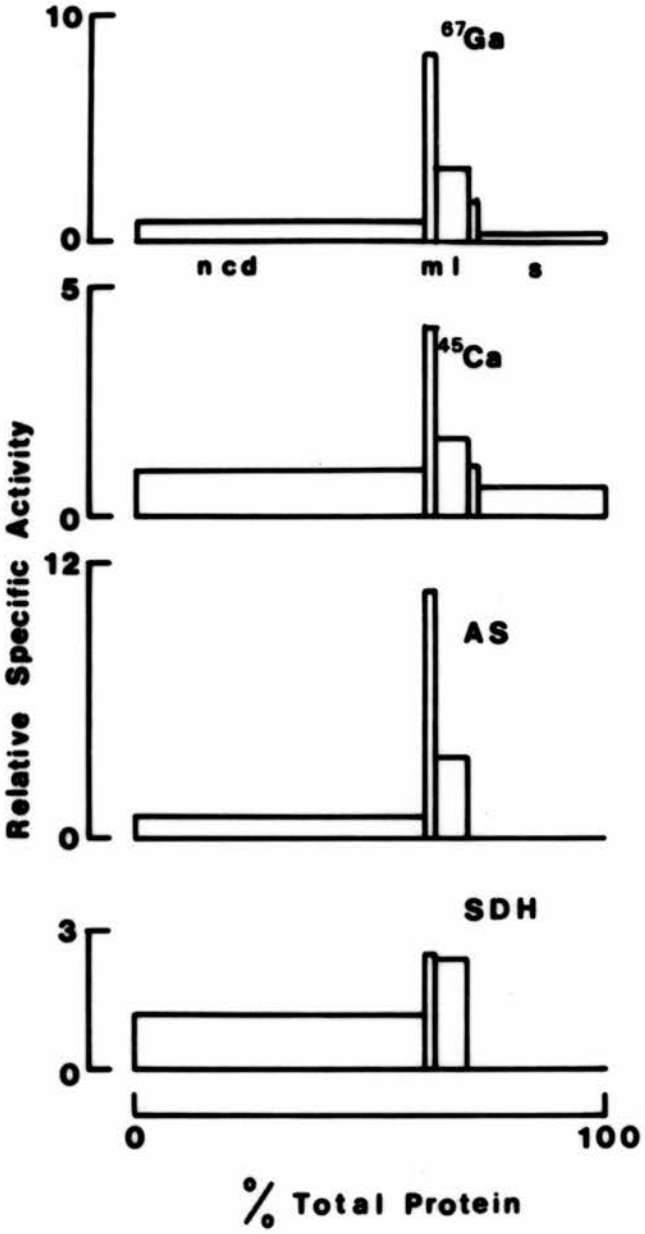


FIGURE **5.5.**

The subcellular distribution of ^{67}Ga, ^{45}Ca, succinate dehydrogenase (SDH), aryl sulfatase in a dog tumour at 72 hr after i.v. injection of the nuclides. ;ions and abbreviations are as in Chart 3 legend. The relative specific rity of all 4 parameters in the nuclei-cell debris fraction is close to ', which probably reflect a high proportion of intact cells in this frac- due to the difficulties of homogenizing this rather fibrous tumour.

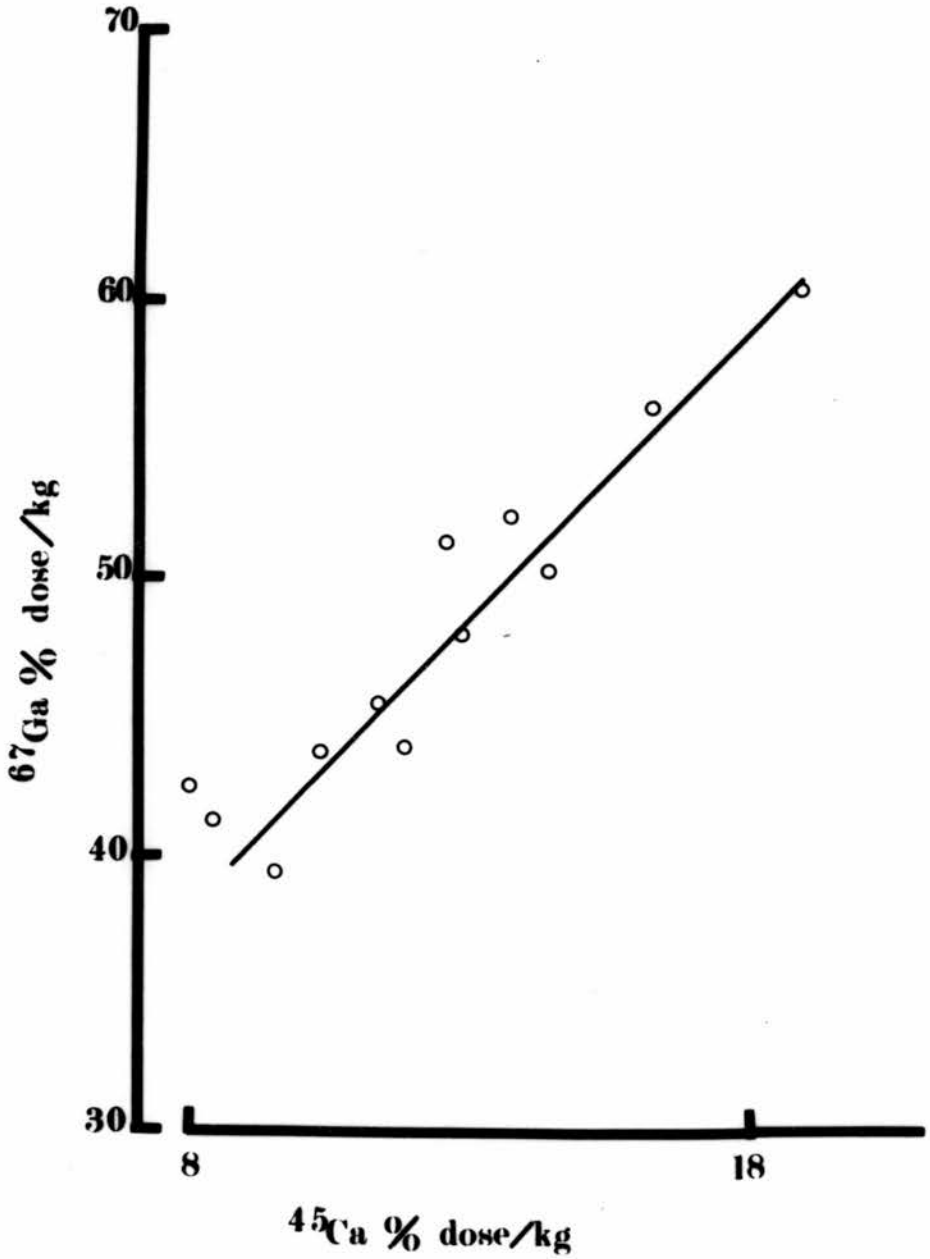


FIGURE **5.6.**

Comparison of the concentrations of ^{67}Ga and ^{45}Ca in 12 samples of breast ue taken from a dog 5 hr after i.v. injection of the nuclides. Correlation 'ficient, 0.948; < 0.001.

nuclei-cell debris fraction. However, this tumour was very difficult to homogenize and the presence of 53 to 75% of the marker enzymes for mitochondria (succinate dehydrogenase) and lysosomes (aryl sulfatase) in this fraction suggests that many of the tumour cells remained unbroken after homogenization and that the ^{45}Ca and ^{67}Ga were probably contained in these unbroken cells. Of the ^{45}Ca and ^{67}Ga not contained in the nuclei-cell debris fraction, most of both nuclides were recovered in the mitochondrial-lysosomal fractions.

Tumour samples were obtained from the dog at 24, 56, and 80 hr. after simultaneous injection of ^{45}Ca and ^{67}Ga. The ^{67}Ga concentration in the tumour was similar at each of the 3 time intervals studied, but the ^{45}Ca concentration in the samples at 56 and 80 hr. was less than one-half that in the sample removed at 24 hr. (Table 5.2). The amount of ^{67}Ga taken up by the lactating mammary gland at 48 hr. was similar to the amount taken up by the tumour (both were around 20% dose injected per kg.), but the amount of ^{45}Ca in the mammary gland was less (7.7% dose ^{45}Ca injected per kg.) than the ^{67}Ga uptake at this time (see Tables 5.1 and 5.2).

The mammary gland and tumour biopsies were cut into 10 to 20 individual samples, and each sample was weighed and assayed for ^{45}Ca and ^{67}Ga. At 5 hr. after injection, there was a very good correlation ($p=0.0001$) between the uptake of ^{67}Ga and ^{45}Ca in the 12 individual samples of mammary gland examined (Figure 5.7), but,

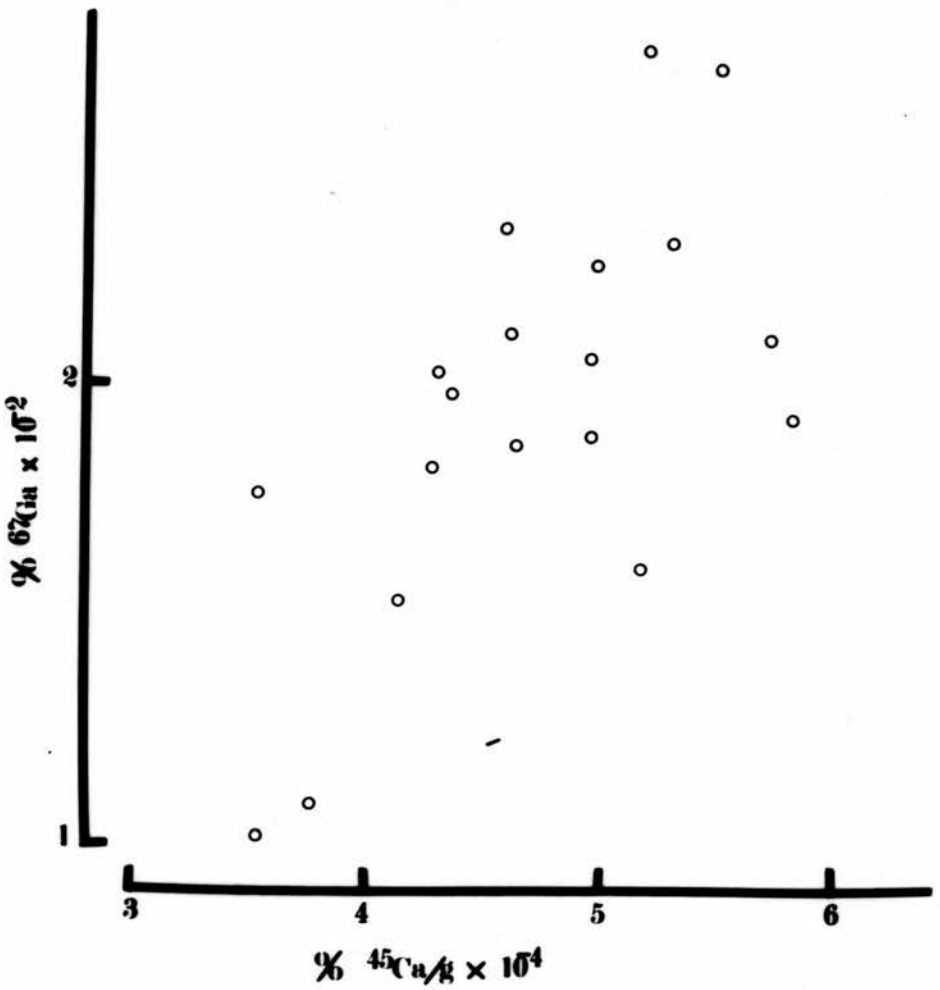


FIGURE **5.7.**

omparison of the concentrations of ^{67}Ga and ^{45}Ca in 19 individual pieces of tumour removed 56 hr after i.v. injection of the nuclides. Correlation icient, 0.190; $p > 0.1$.

48 hr., no similiar correlation was found in 20 tissue samples. A comparison of the concentrations of ^{45}Ca and ^{67}Ga in the individual samples prepared from each tumour biopsy showed no simple correlation between the uptake of ^{45}Ca and that of ^{67}Ga at any of the time intervals studies; the data for the tumour sample removed at 56 hr. are shown in Figure 5.8.

DISCUSSION

This study has examined in more detail the phenomenon of breast imaging occasionally observed when ^{67}Ga-citrate is used to image malignant disease. The case history briefly described in Figure 5.1 shows that breast imaging can occur not only in pregnant and postpartum women, but also occasionally in other patients. We presumed that, in the case described, the hyperprolactinaemia stimulating lactation had been caused by the administration of chemotherapy which contained, among other drugs, chlorpromazine.

These studes on the lactating bitch, which proved an ideal model for collection of blood and adequate quantities of milk, have shown that ^{67}Ga accumulates in both milk and mammary tissue. The ^{67}Ga concentration in milk relative to plasma increases steadily with time, and ^{67}Ga, like ^{45}Ca, is present in milk firmly bound to protein. The concentration of ^{67}Ga in mammary gland tissue is about one-half that found in milk at 5 hr. after injection but, by 48 hr., the concentrations present in milk and in glandular tissues are

approximately equal. It seems, therefore, that it is the uptake of ^{67}Ga in both glandular tissue and milk that causes the visualization of the breasts when scanning is performed at 24 to 48 hr. after ^{67}Ga administration.

The mammary gland biopsies taken at 4 and 48 hr. after ^{67}Ga citrate administration showed little difference in the subcellular distribution (figure 5.3) of the 2 nuclides and, at 48 hr., the highest relative specific activity of both ^{67}Ga and ^{45}Ca was found in the lysosomes.

Similarities in the metabolic behaviour of gallium and calcium have been suggested by Anghileri (1972, 1974) from studies of the subcellular distribution of ^{67}Ga and ^{45}Ca in various experimental tumours and from the displacement of ^{67}Ga from phospholipid and other complexes by excess calcium. The subcellular distribution of ^{67}Ga and ^{45}Ca in the mammary tissue samples examined here appear at first sight to be in broad agreement with the observations of Anghileri. However, the apparent similarities in the subcellular distribution of the 2 nuclides must be interpreted with caution. The subcellular fractionation techniques used by Anghileri and those used in this work, do not produce clear separation between micochondria and lysosomes. Autoradiographic and other studies (D.M. Taylor, unpublished observations) suggest that ^{67}Ga is predominantly associated with lysosomal structures, whereas calcium is known to be associated extensively with mitochondria (Vasington and Murphy 1962). Thus, although most of the total cellular ^{67}Ga

and ^{45}Ca was found in the mitochondrial and lysosomal fractions, the possibility that most of the ^{67}Ga was associated with the lysosomes and most of the ^{45}Ca with the mitochondria cannot be excluded. Perhaps the strongest evidence for an interrelationship between calcium and gallium metabolism comes from the observations regarding milk that showed similar activities of the 2 nuclides, both being wholly associated with the milk proteins.

The lactating mammary gland, although metabolically highly active, is not a rapidly dividing tissue. It is stimulated by the hormone prolactin to produce a variety of proteins, the principle one of which is casein. Calcium metabolism has been studied quite extensively in lactation, especially in domestic animals. There appears to be a common pattern of distribution of minerals in different species between colloidal and soluble phases. For example, two-thirds of calcium in cow's milk is in the colloidal phase as calcium caseinate, phosphate and citrate; one-third is present in the soluble phase as "citrate acid-bound" and ionic calcium (Kon and Cowie, 1961). In this way, normal osmotic equilibrium is maintained in the face of increasing calcium concentration. Calcium is known to be present in the protein membrane of fat globules, and ^{67}Ga concentration may involve a similar mechanism. We found that concentrations of ^{67}Ga and ^{45}Ca in milk were very similar and that there was a positive correlation between the uptake of ^{67}Ga and ^{45}Ca in the multiple pieces of mammary tissue examined at 5 hr. after injection, although, by 48 hr., this

correlation no longer existed. Plasma concentrations of the 2 nuclides were also comparable. On fractionation of the milk, the ^{67}Ga was found to be uniformly distributed throughout the protein fractions, as is calcium. The production of protein by the mammary gland allows cations such as Ca^{2+} and, presumably $^{67}Ga^{3+}$ to be chelated, thereby enabling concentration of minerals to occur while maintaining the osmotic equilibrium.

It was felt that the concentrating mechanism of ^{67}Ga and ^{45}Ca in malignant tumours might be similiar to that in the lactating mammary gland, concentration being allowed to occur by the continual production of binding proteins by the tumour. However, it was not possible to show this in a dog transmissible venereal tumour. This is a spontaneously occurring neoplasm that can be transmitted at coitus and that causes lesions of the penis and vagina. It can be transplanted subcutaneously in dogs, without any form of immunosupression, where it forms large nodules that can be conveniently biopsied. It occasionally metastasizes under these circumstances, but both naturally occurring and transplanted tumours eventually undergo spontaneous remission. It was found that ^{67}Ga concentrated very well in the tumour, but that ^{45}Ca was present in very small amounts. The ^{67}Ga concentration in the tumours was constant over 3 days, but the ^{45}Ca concentration dropped after the first day. Furthermore, there was no correlation between ^{67}Ga and ^{45}Ca uptake in multiple small tumour pieces.

The amounts of ^{67}Ga taken up by this tumour and the lactating mammary gland were similar at 48 hr. In the mammary gland, although there was less ^{45}Ca taken up than ^{67}Ga, the rate of dispersion from the gland was similar. In the tumour, however, ^{67}Ga was retained longer than ^{45}Ca.

These results indicate that, in the lactating mammary gland, ^{67}Ga and ^{45}Ca may follow broadly similar metabolic pathways, but the mechanism of uptake of ^{67}Ga and ^{45}Ca into tumours does not appear to be similar.

4.3 Macrophages and the Uptake of ^{67}Ga in tumours

This section comprised part of a paper given at the 111rd European Congress of Radiology, Edinburgh, 1975.

The paper was entitled "Macrophages and the Uptake of ^{67}Ga-citrate"

INTRODUCTION

The association of ^{67}Ga with "lysosomal-like" structures was first reported by Swartzendruber, Nelson and Hayes (1973) and subsequent reports from various centres have confirmed this observation in a number of other tumours. This group found that ^{67}Ga localization in normal mice occurred mainly in the macrophages of lympho-reticular tissues, thymic epithelial reticular cells, Kuppfer cells, hepatic cells and cells of the proximal convoluted tubules of the kidney. In the leukaemic mice heavy uptake occurred in the thymus and was related to increased numbers of macrophages.

The thesis that ^{67}Ga is taken up by the lysosomal rich phagocytic cells of the reticulo-endothelial system is an attractive one. Recruitment of macrophages is known to occur in certain growing animal tumours (Metcalf, Ishidate and Brumby, 1967) and the idea of ^{67}Ga accumulation within a tumour occurring as a result of the presence of macrophages within the tumour substance would be of immense significance both clinically and from the research point of view.

The recent development of relatively simple techniques has enabled the study of macrophage populations in tumours (Evans and Alexander, 1972). Using these techniques, it is

possible to calculate the macrophage content of transplantable tumours grown in experimental animals and possibly human tumours, although this is proving more difficult due to the fibrous stroma of many human cancers (Gauci and Alexander, 1975).

The MC3 tumour (a sarcoma induced by methylcholanthrene) grows in both Hooded and August rats, but the size of the tumours obtained 10 days after implantation varies considerably. In view of the strong possibility that other factors such as cell proliferation, interstitial fluid volume and vascularity were influencing results, it was decided that the only reasonably accurate comparison of ^{67}Ga uptake would be to examine identical tumours in the same animal strain where the only variable was macrophage content. This opportunity presented itself in the form of the HSBPA tumour, another chemically-induced sarcoma, which after 25 passages exhibited a change in macrophage content to around 50%, whereas earlier passages had a content of around 5 - 10% macrophages. Animals carrying the tumour transplanted at an earlier passage were then compared for ^{67}Ga uptake to animals carrying the tumour transplanted after 25 passages.

Methods

Chemically-induced tumours (MC3 and HSBPA) were transplanted into the hind limbs of two varieties of rats, the Hooded rat and the August rat.

At a time when the tumours had reached the size of some 3-10G, ^{67}Ga citrate was injected intraperitoneally Twenty-four to seventy-two hours later, the rats were sacrificed and the tumour and the liver were dissected out in toto and a muscle biopsy was taken. The tumour, the liver and the muscle biopsy were assayed for ^{67}Ga activity by gamma-ray spectrophotometry in an automatic gamma-ray spectrophotometer.

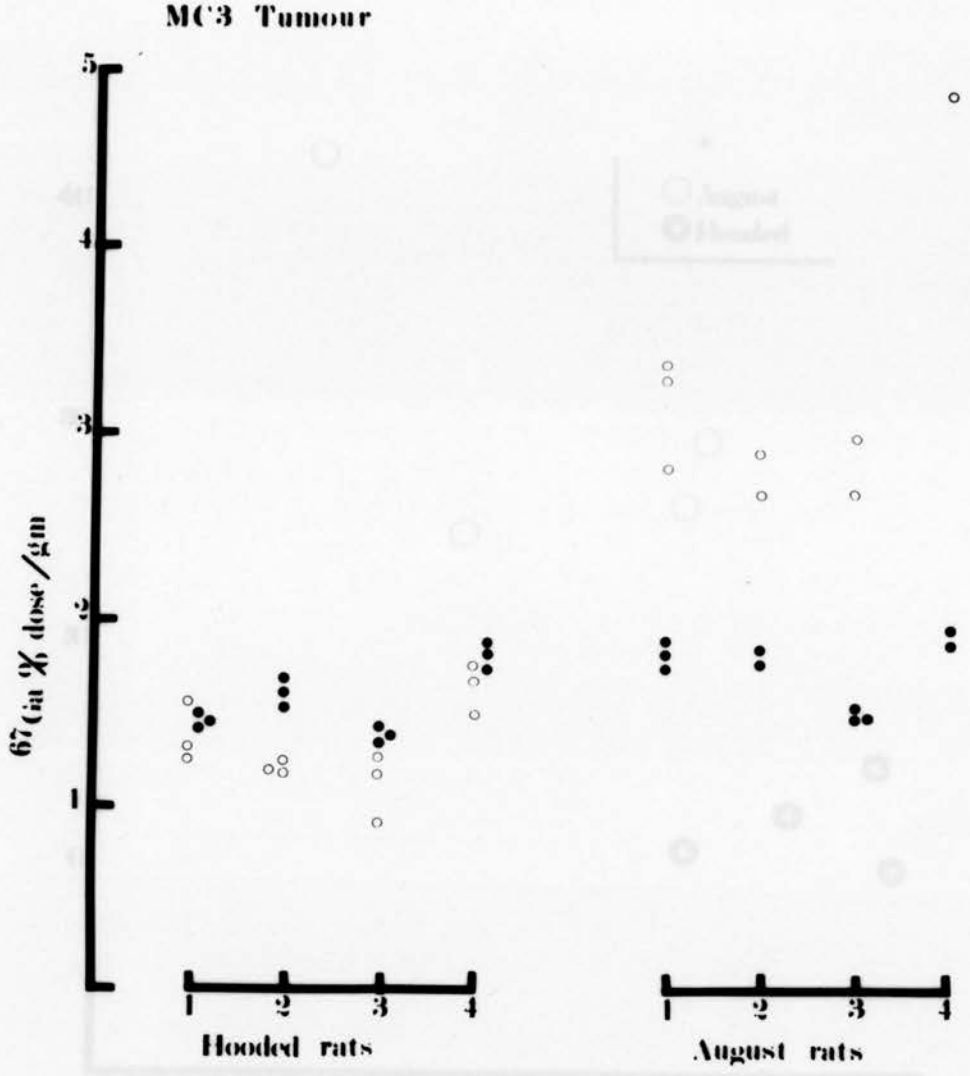
A small sample of the tumour was kept aside for estimation of the tumour macrophage content. The sample was trypsinised for half an hour (0.1G bovine pancreatic trypsin in 100 mls. 0.9% saline with D.N.Aase added) in a continually stirred flask. A drop of the cell suspension thus obtained was pipetted onto a haemocytometer and the total number of large cells were counted. The haemocytometer was then incubated for ten minutes at 37^0 C and the number of glass adherent cells were then counted, using phase contrast microscopy. The number of these cells (macrophages) was then

expressed as a percentage of the total number of cells.

The MC3 tumour was grown in 4 Hooded rats and 4 August rats. The HSBPA tumour was grown in 6 Hooded rats. The HSN tumour, another chemically-induced sarcoma, was grown in 3 Hooded rats for the purposes of comparison. This tumour became infected, but the results are also shown with the HSBPA tumour. One of the HSN rats died as a result of anaesthesia.

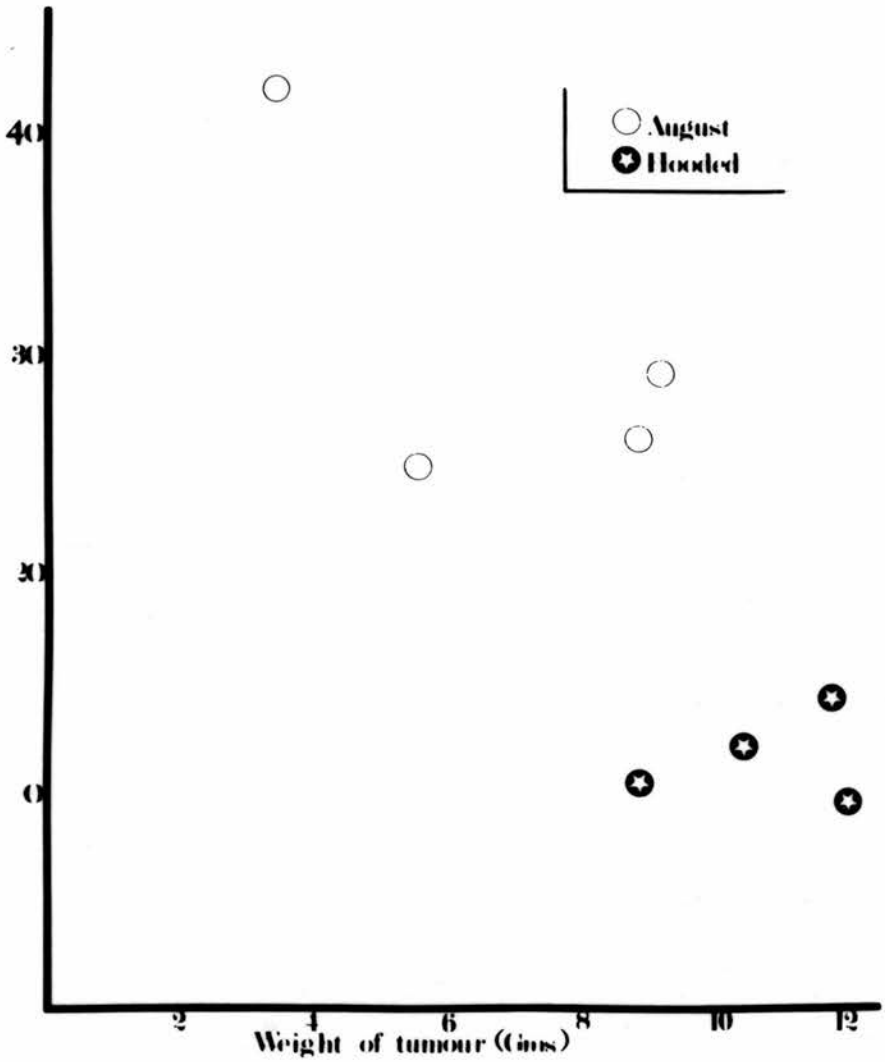
RESULTS

The MC3 tumour grown in the hind legs of Hooded rats has a low macrophage content of around 5%. On the other hand, the same tumour when grown in the hind legs of the August rat, has a macrophage content of beteen 30 - 50%, depending on the passage. At the time of the study, the macrophage content was around 50%. The results of the ^{67}Ga uptake in the MC3 tumour grown in Hooded rats and August rats are shown in Fig. 5.8. The open circles show the ^{67}Ga activity in the MC3 tumour expressed as %dose/Gm wet tissue and the black circles show the ^{67}Ga activity expressed in the same way for the liver. There is a clear difference in the tumour uptake of ^{67}Ga in the two different species of rat, while the liver uptake remains constant. There is almost three times as much ^{67}Ga activity in the high macrophage tumour as there is in the low macrophage tumour. In this experiment, the tumour was weighed in toto, and these results are shown in Figure 5.9. As can be seen, the tumours in the Hooded rats (the low macrophage content group) in general reached a larger size and weight than the tumours in the August rats (the high macrophage content group). Other factors, such as vascularity and interstitial fluid volume may have contributed to the difference in ^{67}Ga uptake.



5.8

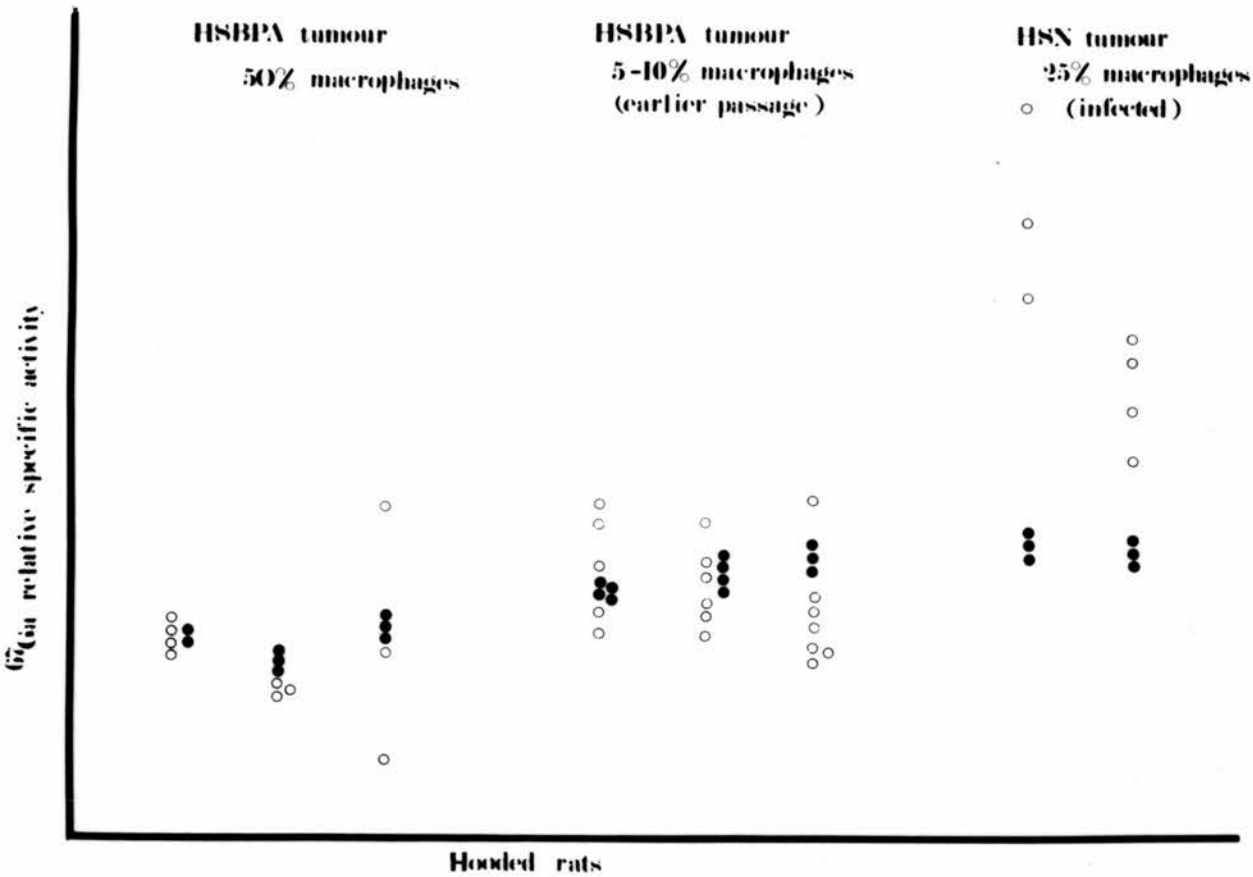
Uptake of ^{67}Ga in tumour (o) compared to liver (•) in the tumour (methylcholanthrene induced) grown in Hooded rats August rats. There is a two-fold increase in ^{67}Ga uptake the tumour of the latter group.



5.9

^{67}Ga uptake (relative specific activity) compared to wet
ıt of the MC_3 tumour grown in August and Hooded rats. The
:umour when grown in August rats is a smaller lighter tumour.

It was therefore decided to examine the ^{67}Ga uptake in the same tumour growing in the same species of animal at times when the macrophage content of the HSBPA tumour varied with the passage of the tumour. ^{67}Ga uptake in the HSBPA tumour grown in Hooded rats was compared between tumours grown after 25 passages when the macrophage content was 50%, and tumours grown at an earlier passage when the macrophage content was 5 - 10%. These results are shown in Figure 5.10. No significant difference in ^{67}Ga uptake was seen in the two groups.



5.10

Uptake of ^{67}Ga (relative specific activity) in tumour (o) liver (•) in the HSBPA tumour grown in Hooded rats. In the columns, the HSBPA tumour has been grown in Hooded rats at ssage when macrophages comprise 50% of the cells within the ur substance. In the middle columns, the HSBPA tumour grown he Hooded rat is at an earlier passage and macrophages comprise 10% of the tumour substance. In the right columns, as a arison, the HSN tumour (grown in Hooded Rats) with 25% macro- es, became infected. ^{67}Ga uptake is greater in this group. e is no difference in uptake in the HSBPA tumour grown in ed rats at the 2 different macrophage contents.

DISCUSSION

In this study, no conclusive evidence was found to support the suggestion that macrophages contributed significantly to the uptake of ^{67}Ga by malignant tumours, using these direct methods.

The association of ^{67}Ga with lysosomal structures has been shown by Swartzendruber, Nelson and Hayes (1973) and confirmed by Hammersley et al (1975), using sub-cellular fractionation techniques. The maximum activity of the nuclide is seen in the fraction containing the highest specific activity of the lysosomal marker enzyme aryl sulphatase.

In attempting to study the role of macrophages in the uptake of ^{67}Ga in tumours, indirect methods have also been used. If macrophages were involved in the uptake of ^{67}Ga, it might be expected that there would be a relationship between the uptake of the nuclide and phagocytic activity as measured by uptake of particulate matter. Hammersley et al (1975) studied mice bearing human tumour xenografts or transplanted murine tumours which were given ^{67}Ga-citrate and $^{99}Tc^{m}$ sulphur colloid intravenously. No consistent correlation was seen in the variety of tumours examined.

In the studies described above, using the MC3 tumour where the macrophage content when grown in the August rat is about 8 times the macrophage content when grown in the Hooded rat, the ^{67}Ga uptake in the August rats is only about threefold the uptake in the Hooded rats. This difference in uptake is probably not significant, as the tumours in the Hooded rats were larger, and larger tumours tend to take up less nuclide than small tumours on a %dose/Gm basis. In the HSBPA tumour grown in Hooded rats, no significant difference in ^{67}Ga uptake was seen in tumours with low macrophage contents or high macrophage contents.

These results confirm the observations with $^{99}Tc^{m}$ and suggest that macrophages do not play a major role in the uptake of ^{67}Ga in tumours.

4.4 ^{67}Ga uptake and Tumour Vascularity

^{67}Ga uptake and Tumour Vascularity

Introduction

It is obvious that for any substance to concentrate within a malignant tumour, the properties of the tumour vasculature and blood-flow are of some importance in determining uptake. It is the relative importance of vascularity in the uptake mechanism which is difficult to assess.

Tumour blood-vessels have formed a recent focus of attention from the work of Folkman and others on tumour angiogenic factor (Folkman 1971). The histological and pharmacological characteristics have also been examined. Neyazaki et al (1970) have described tumour blood vessels as "sinusoidal blood canals consisting of a thin-layered endothelial wall. Endothelial cells of tumour vessels had fenestrations, diaphragms and pores which are also observed in the fenestrated type capillary in inflammation." Their pharmacological characteristics have provided apparently conflicting results although much of the conflict may be due to differing techniques and tumour types used. Zanelli, Fowler and Lucas (1976) have shown that anaesthetics such as Nembutal and Urethane increase

relative perfusion of tumours as measured by uptake of ^{86}Rb, and decreased uptake of ^{125}I H.S.A. The response to adrenaline differs from the response in the normal vascular bed (Abrams 1964).

Blood flow through tumours has been directly measured by Gullino and Grantham (1961). These workers found a greatly reduced blood-flow through tumours when compared to normal tissue and furthermore the blood-flow was independent of tumour size and histology being a fairly constant amount of 0.15 mls± .01/ng tumour.

To investigate the contribution of blood flow, tumour intra-vascular volume and permeability in the uptake of ^{67}Ga into tumours is obviously a major undertaking and the experiments described below were undertaken as a pilot exercise to assess whether any form of relationship of the above factors with ^{67}Ga uptake could be found. It was decided to investigate the permeability of tumour blood vessels to ^{125}I H.S.A.* and its relationship to ^{67}Ga uptake.

*H.S.A. = Human Serum Albumin

METHODS

The Walker-256 carcinoma was implanted into the hind legs of 6 Wistar rats.

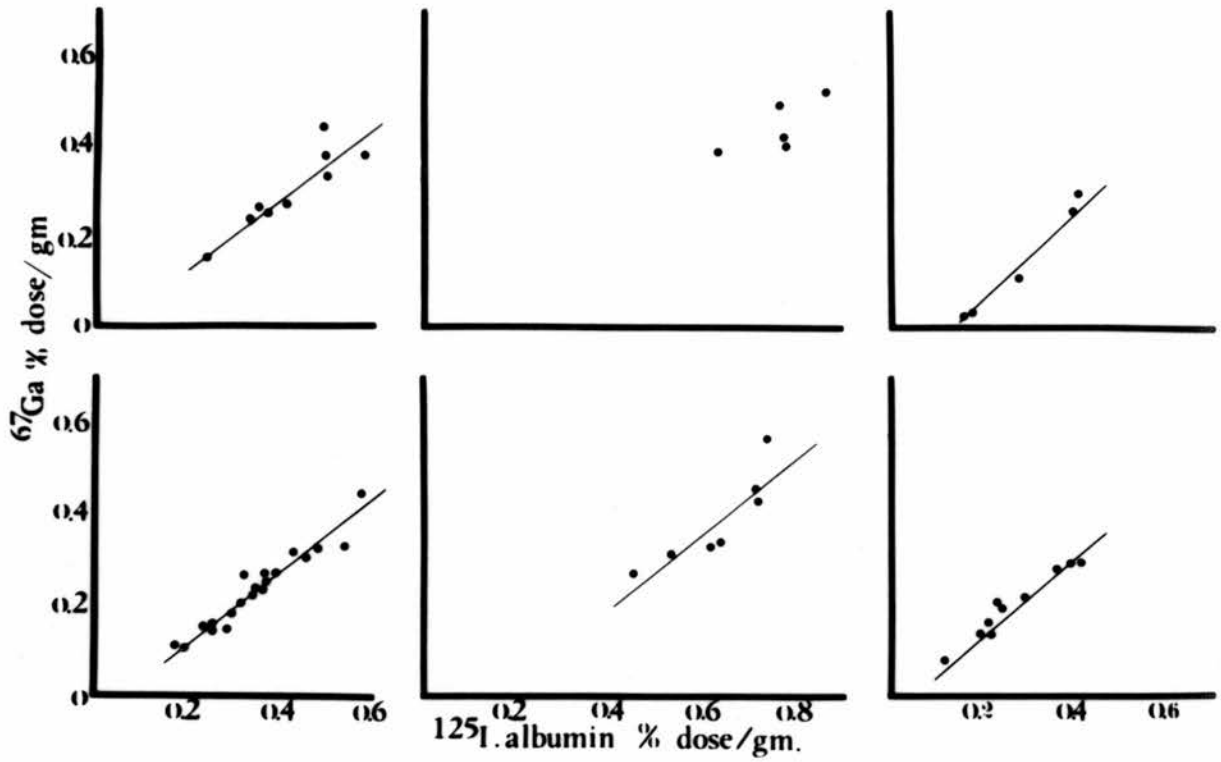
^{67}Ga-citrate (0.1 mls. of saline diluted ^{67}Ga-citrate obtained from Philips-Duphar) and ^{125}I-HSA (0.5 mls of a standard solution of 5 mCi/1ml) were injected intravenously into the tail veins of the animals. The animals were sacrificed at 45 minutes post-injection, and the tumours were dissected out in-toto. The tumours were then cut into multiple small pieces, weighed and assayed for ^{67}Ga and ^{125}I on an automatic gamma-spectrometer using appropriate channels for ^{67}Ga and ^{125}I. An aliquot of the solutions injected was assayed as a standard.

RESULTS

The results are expressed as % dose injected/gm. tissue. Statistics were performed by standard Chi-squared analysis.

Figure 5.11 shows the results of ^{67}Ga uptake in multiple pieces of Walker 256 carcinoma in 6 Wistar rats sacrificed 45 mins. after injection of ^{125}I. H.S.A. and ^{67}Ga-citrate. There is a highly significant positive correlation of uptake of the two radionuclides in 5 of the 6 animals. ($P > 0.01$).

Comparative uptake of ^{67}Ga and ^{125}I.albumin in Walker 256 in rats at 45 minutes.



5.11

There is a statistically significant correlation ($p<0.05$) between ^{67}Ga uptake and ^{125}I. labelled albumin in 5 of 6 Wistan at 45 minutes post-injection.

DISCUSSION

The results of these preliminary experiments suggest that soon after injection of ^{67}Ga citrate, the intra-tumour distribution of ^{67}Ga closely resembles the distribution of ^{125}I H.S.A. There was a strong positive correlation in distribution of the two nuclides within the tumour at 45 minutes. Furthermore, the absolute amounts of the two nuclides were similiar at around 0.3% dose injected/gm. for the Walker-256 carcinoma. It is reasonable to suppose therefore that protein-bound ^{67}Ga, either as ^{67}Ga-transferrin or loosely bound to albumin or globulin, enters the tumour interstitial fluid via fenestrations and pores in the loose network of endothelial cells which form the tumour vasculature, in the same way as ^{125}I H.S.A. Thereafter, other factors must take over. This might be explained by the ^{125}I H.S.A. becoming evenly dispersed throughout the interstitial fluid of the tumour and, in contrast, the ^{67}Ga becoming attached to binding sites fairly rapidly and thereby not dispersing evenly. It is known that ^{67}Ga activity is associated with viable tumour tissue, the necrotic areas having low ^{67}Ga uptake. In the Walker-256 carcinoma much the same total amount of ^{67}Ga and ^{125}I H.S.A. as % dose injected, was taken up at 45 minutes.

The question as to whether tumour vascularity is a major contributing factor to the tumour image obtained by Gallium scanning must await further studies, in particular, a measurement of total uptake of the two nuclides over a series of time intervals.

It seems likely that once ^{67}Ga has entered the tumour interstitial fluid, secondary uptake mechanisms come into play, allowing thereby the accumulation and retention of the nuclide in a similar way to ^{125}I H.S.A. where initial permeation of the tumour vasculature is followed by pinocytosis of the nuclide complex by the tumour cells (Raimondi 1967). In summary, therefore, tumour vascularity plays a part in the uptake mechanism of ^{67}Ga into tumours but it is not known whether it is a major factor in its retention.

CONCLUSIONS

There can be no doubt that the technique of whole body scanning using tumour imaging radiopharmaceuticals is potentially one of the simplest and most accurate methods of demonstrating the local site and distant spread of malignant tumour. However, it must be obvious from this thesis that at present, although relative simplicity of technique has been achieved, we cannot yet consistently and reliably detect tumour deposits less than 2 cm. in diameter. Occasions when present radiopharmaceuticals and apparatus reveal disease not shown by other means are few, but this should not detract from the important advantage of ease of technique when compared to other diagnostic methods.

From the survey of a substantial number of clinical reports, it is concluded that no one tumour imaging agent is vastly superior to another. There are striking similarities in the kinds of tumours demonstrable by these agents and they can all exhibit uptake into inflammatory exudates. (An explanation for this may be the presence of the "fenestrated" capillary in neoplasms and inflammation. This "leaky" capillary is also present in endocrine glands, choroid plexus and intestinal villi - regions where ^{67}Ga uptake may be higher than normal. Other areas of high nuclide uptake such as the liver, spleen and bone marrow have sinusoidal vascular channels, consisting of a discontinuous type of capillary with large pores and a defective basement membrane).

^{67}Ga-citrate is probably the most widely used tumour imaging

agent. The main reason for this is that, for scanning, it has very suitable physical properties and an acceptable radiation risk for use in patients. The clinical scans obtained appear to be better with ^{67}Ga-citrate than with other agents, but there is only limited comparative evidence to support this view. The comparative study between ^{111}In-bleomycin and ^{67}Ga-citrate described in this thesis is one of the few controlled studies reported in the literature and one of the largest. It is an obvious point, but one worth emphasizing, that in the detection of small differences, a comparative trial of similar design to the one described is essential. In this trial, it was readily proven that ^{67}Ga-citrate was more valuable than ^{111}In-bleomycin in the detection of lesions above the diaphragm. One of the interesting points to come out of this trial was that the detectable differences were not an "all-or-none" imaging difference, but one of degree. Very few lesions imaged with one radiopharmaceutical and not the other. In the comparative study of ^{57}Co-bleomycin with ^{67}Ga-citrate, the disadvantage of difficult radioactive waste disposal was greater than any possible improvement in imaging quality.

In the subsequent clinical studies, by examining a specific malignancy in depth an attempt was made to elucidate common factors for adequate tumour uptake of ^{67}Ga. Hodgkin's disease, non-Hodgkin's lymphoma and carcinoma of the bronchus are imaged well by a number of radiopharmaceuticals, including ^{67}Ga-citrate. It was found that seminoma of the testis produced consistently excellent scans; tumour

deposits measuring 1.5 cm. in diameter could be clearly imaged. This malignancy, curable by modern radiotherapy, provided a clear indication for Gallium scanning, since tumour deposits, not found by other investigative techniques, were frequently found. On the other hand, Gallium scans in Hodgkin's disease often recorded disease sites which had been found by other techniques. There is no good evidence of uptake of other radiopharmaceuticals in seminoma of the testis since they have been infrequently scanned in reported series. Seminoma deposits were invariably picked up by Gallium scanning whereas teratoma deposits, although often being detectable, were not consistently detectable. One patient described, who had a combined testicular tumour showed positive Gallium scans during the seminoma phase of his illness and negative scans during the teratoma phase, suggesting that the biological change from "seminoma-predominance" to "teratoma predominance" had changed the properties of uptake of radionuclides.

In the retrospective analysis of Gallium scans in Hodgkin's disease, the main conclusion drawn from the many graphs and tables presented, was that patients who were approaching the end of their disease often showed marked uptake of ^{67}Ga in tumour sites. The possible relationship of level of initial uptake of ^{67}Ga to prognosis is very suggestive and a prospective study would be the next obvious step.

From the aspect of future development of this diagnostic technique, one must consider carefully the aims in tumour diagnosis.

In most clinical series, the majority of cases are comprised of large tumours which have been detected by other methods and then scanned to see whether or not uptake occurs. In these cases, presumably the aim of the scan is to differentiate between benign and malignant tumours. However, it is clear that the tumour imaging radionuclides are not specific for malignant lesions, and it is likely to be difficult to improve this specificity for reasons to be discussed later. Furthermore, the histological diagnosis of a lesion will always in the end be decided by a histologist. Therefore, there are two areas of clinical interest where research should be channelled. The first area is in the detection of small tumour deposits and the second is in the monitoring of the activity of established malignant disease. It is unlikely that radioactive tumour imaging agents will ever be used as screening tests for the early diagnosis of malignant lesions because of the radiation hazards involved, but the ability to detect small tumour metastases in the investigation of the patient with histologically proven cancer would obviously be valuable. This attempted goal must be viewed in the light of recent advances in other diagnostic methods, such as ultrasonography. This technique is highly competitive in certain areas, particularly the abdomen, but in contrast to radionuclide tumour imaging, ease of whole body screening is not a feature of ultrasonography in its present state of development. In order to detect smaller lesions by means of tumour imaging radionuclides one will need agents which give more favourable tumour:background ratios, whether this is

achieved by increasing the concentration of the agent within the tumour or by lowering the background activity.

It is proving difficult to account for the mechanism of uptake, accumulation and retention of these nuclides on a simple basis. It is likely that the permeability characteristics of tumour blood vessels are a significant factor in ^{67}Ga uptake, allowing the radionuclide to come into contact with tumour cells after which other accumulator mechanisms take over. The results obtained in the Walker-256 carcinoma with ^{67}Ga and ^{125}I HSA would be consistent with this idea.

These accumulation mechanisms may vary with the predominant cell type in the tissue being studied. Merz et al (1974) have shown that in thymus dependent lymphocytes, both unstimulated and stimulated by PHA, ^{67}Ga associated tenaciously with the plasma membrane. On the other hand, in the lactating mammary gland (section 4.1), similarities were found in the accumulation of ^{45}Ca and ^{67}Ga in breast tissue. These similarities were not found when tumour tissue was studied. The thesis that tumour macrophages play a significant part in ^{67}Ga uptake and retention in tumours was not confirmed (Section 4.3).

An important need is to develop a reliable method of monitoring the activity of malignant disease after treatment: we have few methods of doing this apart from clinical examination and radiography, both of which have their limitations. To achieve this

purpose several obstacles must be overcome. Firstly, we need to know the effect of treatment on the uptake of the agent and secondly we must know as precisely as possible why these agents accumulate in tumours. The third obstacle will be rather more difficult to overcome. This is to produce a tumour imaging agent which is more specific for malignant disease than any other available at present. The reason for this difficulty is simply that there is no known constant feature of malignant disease which is not at some time observable in normal tissues.

However, there can be no doubt that the task is worth the effort when one considers the few methods we have for the study of the behaviour of tumours in humans. The study of radioactive tumour imaging agents involved in some intrinsic and understood part of the malignant process would broaden our knowledge of human cancer.

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